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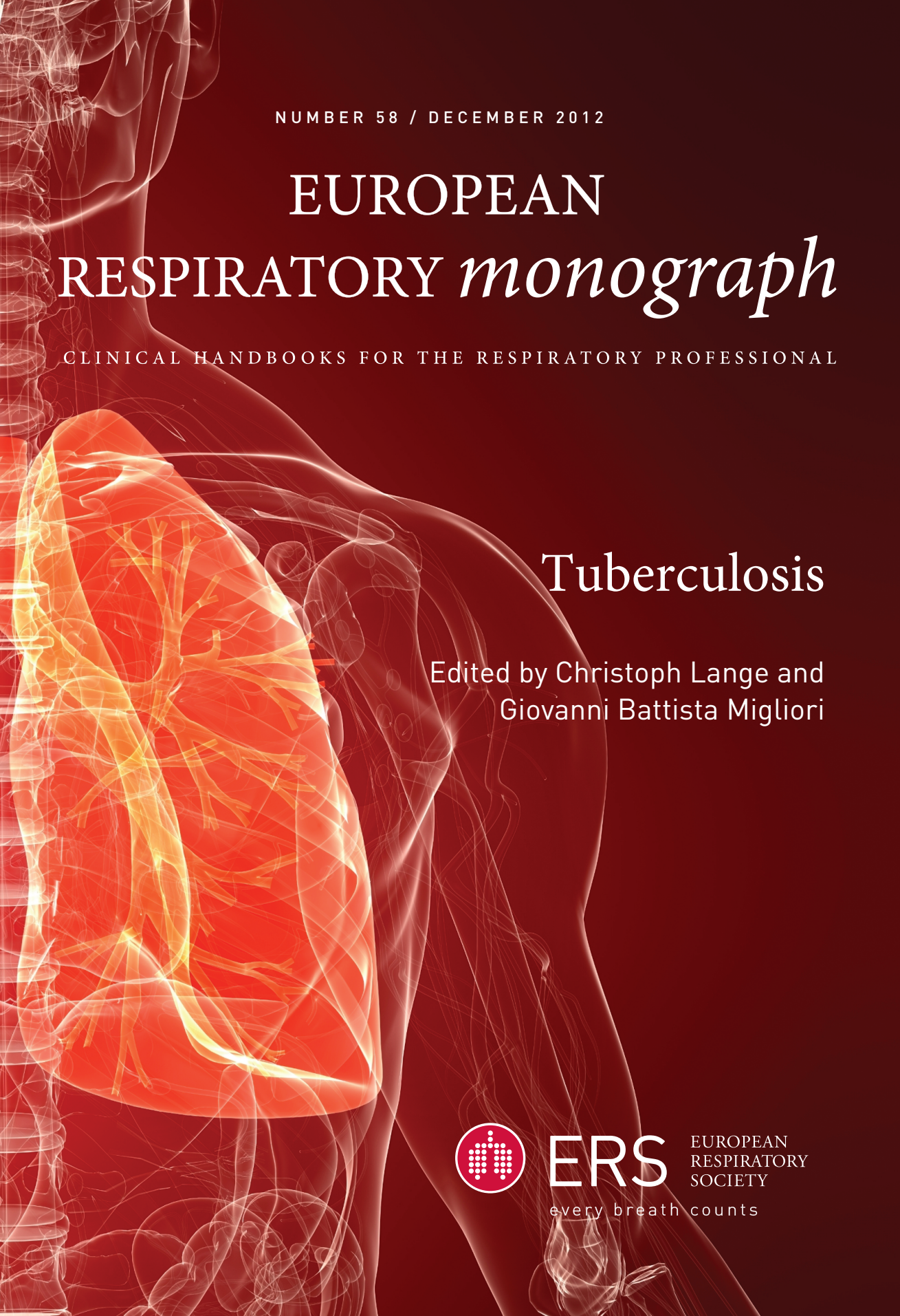

Tuberculosis

Edited by Christoph Lange and Giovanni Battista Migliori

ERS EUROPEAN RESPIRATORY SOCIETY
every breath counts

European Respiratory Monograph 58, December 2012

Tuberculosis

Edited by
Christoph Lange and Giovanni Battista Migliori

Editor in Chief
Tobias Welte

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This book is one in a series of *European Respiratory Monographs*. Each individual issue provides a comprehensive overview of one specific clinical area of respiratory health, communicating information about the most advanced techniques and systems required for its investigation. It provides factual and useful scientific detail, drawing on specific case studies and looking into the diagnosis and management of individual patients. Previously published titles in this series are listed at the back of this *Monograph*.

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Preface

With the introduction of tuberculostatic antibiotics and the improvement of living conditions in developed countries, it was generally felt that the problem of tuberculosis (TB) had been overcome. However, the persistent problems caused by the disease that were seen in developing countries were not really noticed beyond expert circles. With the spread of AIDS in the 1980s, TB returned to the forefront of attention as one of the major opportunistic infections in AIDS patients. In the years that followed, the first reports of difficult-to-treat multidrug-resistant (MDR)-TB were published. In the beginning, reports were limited to countries with a high AIDS prevalence; however, after the collapse of the Soviet Union and the opening of the former Communist countries to the West, the full extent of MDR-TB became clear.

The number of presentations that focussed on TB at the 2012 European Respiratory Society (ERS) Congress in Vienna was truly amazing. Many of the contributions reported increasing MDR in different parts of the world. A recently published study from eight countries, including Estonia, Latvia and Russia, demonstrated a dramatic increase in extensively drug-resistant (XDR)-TB [1]. Previous treatment with second-line anti-TB drugs was shown to be the main risk factor. The development of new drugs has been significantly slower than the increase in resistance; 10 years on, there have been no advances specifically for this area. Only antibiotics approved for other indications, such as fluoroquinolones and linezolid, have provided new treatment options. It is thanks to the commitment of the World Health Organization (WHO) and the support of private foundations (primarily the Bill and Melinda Gates Foundation) that there has been research into new tuberculostatic substances. Some of these are currently being tested in early clinical trials, giving hope of improved treatment options in the future.

It is clear, then, that TB is once again a subject of intense interest. This edition of the *European Respiratory Monograph* (*ERM*) is therefore particularly topical, as it presents current scientific discussion in the practice of TB diagnosis and treatment. I would like to congratulate the Guest Editors of this issue of the *ERM*, who have brought together contributions from the best experts in the field. This book provides an excellent overview of the topic, and should be of interest to general practitioners and chest physicians, as well as those in industry and in public health who are faced with the problem of TB everyday.

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Guest Editors

Christoph Lange

Christoph Lange is a physician and biologist. He was appointed as Professor of Medicine at the University of Lübeck (Lübeck, Germany) in 2009 and is currently the Leading Attending Physician at the Medical Clinic/Head of the Division of Clinical Infectious Diseases at the Research Center Borstel (Borstel, Germany). Since 2005, he has been Co-Chair of the Center of Infectious Diseases (DGI) at the Research Center Borstel and the University of Lübeck, and he is Vice-Chair of the Center of Infection and Inflammation at the University of Lübeck (ZIEL). Following clinical training in Cape Town (South Africa), Cleveland, OH (USA), and hospitals in Germany, he is board certified in internal medicine, pulmonary and critical care medicine, infectious diseases, allergology and sleep medicine. His research focus is on tuberculosis (TB), in which he has been principle investigator of national and international multicentre studies, and a coordinator of consensus documents. C. Lange is Head of the Respiratory Infection Assembly (Assembly 10) of the European Respiratory Society (ERS) (2011–2014) and was previously (2008–2011) chair of the Tuberculosis Group (Group 10.2) of this Assembly. He is the Founding Chair of the Tuberculosis Network European Trials Group (TBNET), which he headed from 2006 to 2012. Since 2011, he has been a member of the steering committee of the Mycobacteriology Study Group (ESGMYC) of the European Society for Clinical Microbiology and Infectious Diseases, and from 2009 to 2012, he was the chair of the Mycobacteriology Study Group of the DGI. C. Lange is an Associate Editor of the *International Journal of Tuberculosis and Lung Diseases*, an Associate Editor of *Infection* and Section Editor for Respiratory Infections of the *Clinical Respiratory Journal*. He has been a Guest Editor for TB series in the *European Respiratory Journal* (*ERJ*) and *Respirology*. C. Lange was appointed a Visiting Professor at the University of Medicine and Pharmacy of the Republic of Moldova in Chisinau (Moldova) in 2011 and a Fellow of the Infectious Diseases Society of America.

Giovanni Battista Migliori

Giovanni Battista Migliori, MD, FRCP (Lond) is Director of the World Health Organization (WHO) Collaborating Centre for TB and Lung Diseases (Fondazione S. Maugeri, Tradate, Italy), a Professor in the Applied Health Sciences Department of the University of Pavia (Pavia, Italy) and a Visiting Professor at the Mayo Clinic (Rochester, MN, USA).

He originally specialised in respiratory medicine in Pavia and then in medical statistics and epidemiology. He has developed global experience in TB, TB/HIV and HIV/AIDS care and control in Africa (Benin, Burkina, Democratic Republics of Congo, Egypt, Ethiopia, Mozambique and Tanzania), Asia (India and Thailand), Europe (Croatia, Estonia, Kosovo, Latvia, Moldova, Romania, Russia, Turkey and Ukraine) and Latin America (Mexico). G.B. Migliori has served as ERS Tuberculosis Group Chair (Group 10.2), as Head of the Respiratory Infections Assembly (Assembly 10) (2008–2010), Long Range Planning Committee Chair (2011–2013), and is presently the ERS WHO and European Centre for Disease Control Liaison Officer and Secretary General Elect.

Since 2007, he has been an Associate Editor of the *ERJ* and since 2011 of the *International Journal of Tuberculosis and Lung Diseases*, having previously served in the same capacity at *BMC Infectious Disease*.

He is an author of more than 250 publications (including the 1999 ERS Guidelines on TB Management and the 2012 EU Standards for TB care) with a personal H-Index of 38 and average impact factor of more than 220 over the last 3 years. As Guest Editor, he was responsible for two *ERJ* TB Series (2010 and

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2011) and the *Respirology* TB Series (2010), and has collaborated in the *Lancet* TB Series (2011).

Within the International Union against Tuberculosis and Lung Disease (IUALTD), he was Secretary General and President (2008–2009) of the Europe Region.

He currently serves WHO as a member of STAG (Scientific Technical Advisory Group, 2010–2012), WHO EURO as a member of TAG (Technical Advisory Board) as well as the GFATM (Global Fund Against AIDS, Tuberculosis and Malaria) as Rotating Member, TRP (Technical Review Panel and Consultant), the GDF (Global Drug Facility) and the European Commission (TACIS programme, Expert Group and the Scientific Committee of EDCTP (European and Developing Countries Clinical Trials Partnership)).

G.B. Migliori is former Secretary of TBNET (2006–2011).

Introduction

Christoph Lange[*,#,¶] *and Giovanni Battista Migliori*[+]

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Although the global incidence of tuberculosis (TB) is slowly declining, the disease still remains one of the leading causes of morbidity and mortality worldwide. In many regions of the world, including parts of Europe, the high incidence of TB is an indicator of poverty and healthcare inequalities. In countries with a low incidence of TB, the disease is mainly related to risk group; for example, recent close contacts of infected index patients, migrants from high-incidence countries, HIV-infected individuals, and those with other immunodeficiencies and co-morbidities. Targeting these groups in order to prevent TB is particularly important if the ultimate goal of eliminating the disease is to be achieved.

The emergence of antimicrobial drug resistance in *Mycobacterium tuberculosis* strains with multidrug- (MDR) and extensively drug-resistant (XDR) profiles has become a major obstacle to achievement of the Millennium Development Goal of TB, which aims for the elimination of the disease by 2050. However, for the first time in many centuries, new drugs for the treatment of TB will soon become available. These new weapons must be used wisely.

There may be a further barrier to disease elimination: medical students and even residents of internal medicine from industrialised countries may complete their education without seeing a single patient with TB. This lack of experience is reflected in the diagnostic delay seen in these countries.

However, important advances have been achieved in the prevention, diagnosis and treatment of TB in recent years. This has been due to the hard work and dedication of leaders in the field, many of whom have kindly agreed to contribute to this issue of the *European Respiratory Monograph* (*ERM*). We are very grateful for their commitment to the field and for their support in the compilation of this publication.

This issue of the *ERM* includes up-to-date information on TB epidemiology, as well as control and elimination strategies. TB prevention, including the latest developments in novel vaccine research, and recent advances in the diagnosis and treatment of latent infection with *M. tuberculosis*, are also presented. The issue also covers state-of-the-art diagnosis of TB and the treatment of active TB in patients from different risk groups, including cases of *M. tuberculosis* drug resistance and complications of therapy.

We hope that this issue of the *ERM* will prove a useful and valuable reference source to our colleagues, and will inspire young clinicians and scientists to enter this fascinating field.

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Chapter 1

Microbiology of *Mycobacterium tuberculosis* and a new diagnostic test for TB

Vera Katalinić-Janković[*], *Lucinda Furci*[#] *and Daniela Maria Cirillo*[#]

SUMMARY: Tuberculosis (TB) has been one of the most important human diseases for centuries now. It is mainly caused by *Mycobacterium tuberculosis*, a highly elusive bacillus. This intracellular pathogen does not possess the classic bacterial virulence factors. However, *M. tuberculosis* efficiently evades the immune response by complex and manipulative mechanisms, which enable survival for as long as decades. The fight with such a smart rival gives rise to the necessity for early diagnosis and appropriate treatment. The ability to rapidly detect *M. tuberculosis* in clinical specimens, as well as drug resistance, is essential for the appropriate treatment of TB patients and the prevention of spread of drug-resistant strains. New molecular tools are now used in many countries as part of a standard laboratory diagnosis. It is clear that important advances in TB diagnosis have recently been made and potentially useful new tools are emerging. Nevertheless, there is still a lot to be done, especially in high-burden countries where fast identification and early treatment are needed.

KEYWORDS: Diagnostic tools, drug resistance, immunity, latency, *Mycobacterium tuberculosis*, pathogenesis

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In 1882, Robert Koch discovered *Mycobacterium tuberculosis*, the bacillus responsible for tuberculosis (TB), thus identifying TB as an infectious disease [1]. This discovery led shortly thereafter to the identification of methods to stain bacilli in clinical specimens, making the organisms identifiable with the use of light microscopy. Such was the birth of TB diagnostics and of microbial diagnostics in general. The name *Mycobacterium*, which means "fungus bacterium", was introduced in 1896 [2]. It describes the way that the tubercle bacillus grows on the surface of liquid media as mould-like pellicles [2]. These aerobic, asporogenous rods have been referred to as the "ducks of the microbial world" due to their thick, waxy outer coating. The genus *Mycobacterium* comprises a number of aerobic bacteria and is the only member of the family *Mycobacteriaceae*, sharing an unusually high genomic DNA G+C content (62–70%) and the production of mycolic acids with the closely related

genera *Nocardia* and *Corynebacterium* within the order *Actinomycetales*. The most important member of the genus, *M. tuberculosis*, is an intracellular pathogen that does not possess the classic bacterial virulence factors such as toxins, capsules or fimbriae. Several structural and physiological properties of the bacterium are recognised for their contribution to virulence and pathology of the disease. *M. tuberculosis* is an aerobic, non-spore forming, non-motile bacillus with a high cell wall content of high molecular weight lipids, which comprise approximately 60% of the cell wall structure. Due to this cell wall composition, mycobacteria stain poorly with Gram stain but are described as acid-fast, as once stained with hot carbol-fuchsin it resists decolourisation with acidified organic solvents (Ziehl–Neelsen stain) [3]. The high lipid concentration in the cell wall accounts for its impermeability and resistance to antimicrobial agents, and resistance to killing by acidic and alkaline compounds in both the intra- and extracellular environment. *M. tuberculosis* has the ability to form serpentine structures (cords). The cord factor is primarily associated with virulent *M. tuberculosis* strains. Although its exact role in *M. tuberculosis* virulence is unclear, it is known to be toxic to mammalian cells and to be an inhibitor of polymorphonuclear leucocyte migration. *M. tuberculosis* grows successfully under aerobic conditions but it is also able to survive in oxygen-deprived environments. *In vivo*, *M. tuberculosis* grows better in tissues with a high oxygen content, such as the lungs. The bacillus divides every 20–22 hours, and this slow replication rate and the ability to persist in a latent state means that individuals infected with *M. tuberculosis* require long periods of drug and preventive therapies.

TB is caused by *M. tuberculosis* and TB complex members (*Mycobacterium bovis, M. bovis* bacille Calmette–Guérin (BCG), *Mycobacterium africanum, Mycobacterium canettii, Mycobacterium pinnipedii and Mycobacterium microti*) and is one of the most intensively studied human diseases. It can target practically any organ of the body and clinical microbiological studies have been performed for decades. Humans are the only reservoir for the *M. tuberculosis* species, although many animals are also susceptible to infection [4]. *M. bovis* was responsible for about 6% of all human deaths in Europe before the introduction of milk pasteurisation and attenuation of a laboratory strain of *M. bovis* led to the development of the BCG vaccine in 1921.

In the 1950s, it became clear that other *Mycobacterium* spp. in addition to those causing TB and leprosy were also human pathogens. In 1959, RUNYON [5] proposed a classification of these non-tuberculous mycobacteria (NTM) into four major groups, based on growth rates and colony pigmentation. NTMs are generally free-living organisms that are ubiquitous in the environment around the world, and can be found in deserts, under rocks and among dried roots of vegetation [6]. Their optimal habitat in the environment is close to fresh water, both flowing and static. Currently, more than 150 NTM species have been identified. Phylogenetic trees are available that depict genetic relatedness based on homology of the 16S ribosomal RNA (rRNA) gene sequence. Mycobacteria that have highly homologous rRNA sequences are closely related and are on neighbouring branches of the tree [7]. The mycobacterial phylogenetic tree can be further subdivided into fast- and slow-growing bacteria. The fast growers form colonies on selective media in less than 7 days, whereas the slow growers take more than 7 days. In addition, within the genus *Mycobacterium* a number of species are grouped into complexes (*e.g. Mycobacterium avium* and *M. tuberculosis* complexes) that include bacterial species that have a high degree of genetic similarity and cause similar disease syndromes. The *M. tuberculosis* complex species share 99.9% sequence identity and probably evolved from a single clonal ancestor.

Advances in mycobacterial genomics are providing evidence that the amount of sequence variation in the *M. tuberculosis* genome might have been underestimated and that some genetic diversity does have important phenotypic consequences. Studies of the phylogeny and biogeography of *M. tuberculosis* have revealed six main strain lineages that are associated with particular geographical regions [8].

Pathogenesis and immunity

Early steps of infection

M. tuberculosis is a highly successful bacterial pathogen that mainly targets host macrophages, key mediators of both innate and adaptive immune response. In lung infections, *M. tuberculosis* is

typically inhaled into the body, passes through the airways and reaches the alveolar space. Here, it interacts with dendritic cells [9, 10], alveolar macrophages and pulmonary epithelial cells, but its optimal hosts are alveolar macrophages and other mononuclear phagocytes [11]. *M. tuberculosis* gains entry into alveolar macrophages through receptor-mediated phagocytosis, a normal feature of the innate immune system. Two main routes are exploited: bacterial cell surface molecules activate complement proteins present in the alveolar space, which are then recognised by complement receptors on macrophages; or alveolar macrophages recognise bacterial mannose residues (particularly mannose-capped lipoarabinomannan), directly through binding with macrophage mannose receptors (fig. 1) [12]. Alveolar macrophages are attractive targets for *M. tuberculosis* because they are adapted to the task of removing small airborne particles through phagocytosis, and

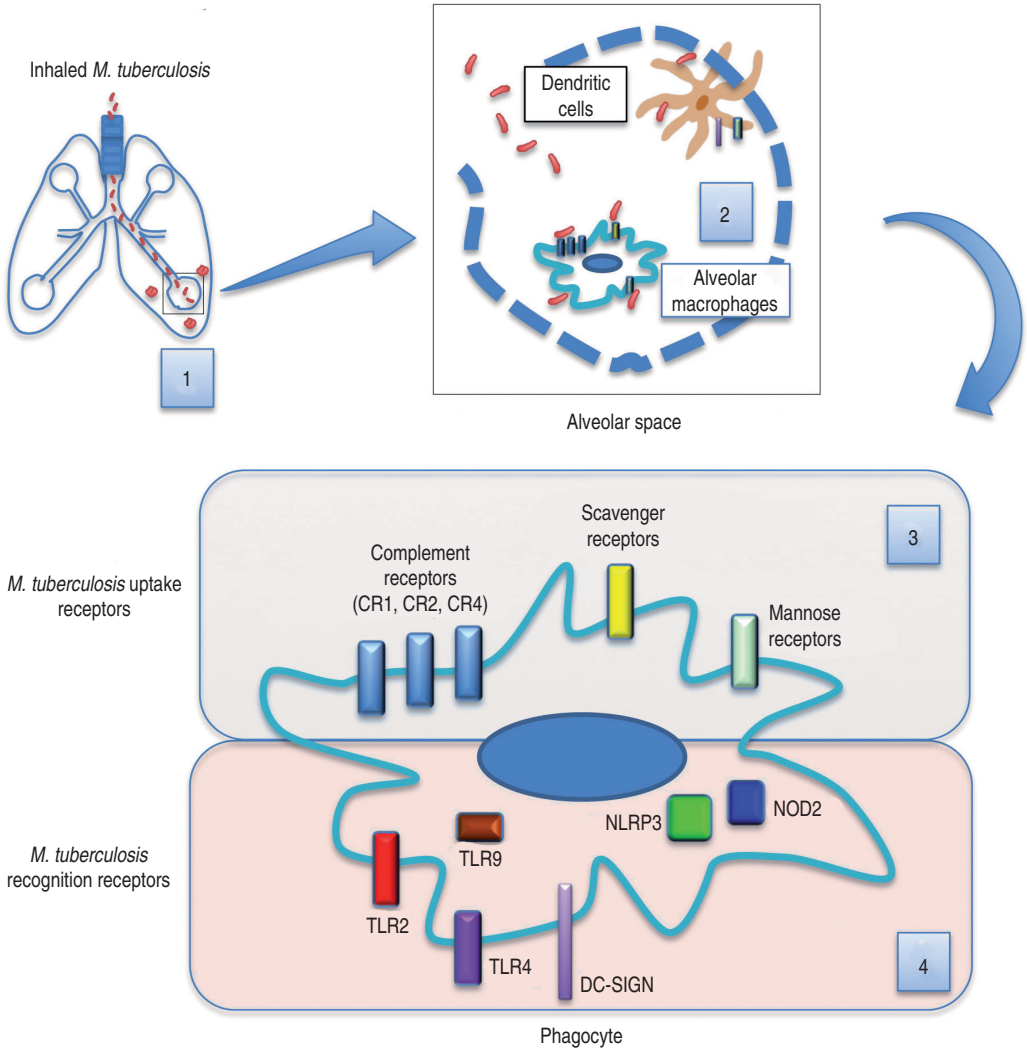

Figure 1. Early steps of phagocyte infection. 1) *Mycobacterium tuberculosis* is inhaled through the airways and travels all the way to the alveoli of the lower portion of the lungs where it establishes stage I infection. 2) In the alveolar space, mycobacteria are actively phagocytosed by resident macrophages and dendritic cells. 3) Complement receptors (CR) are primarily responsible for uptake of opsonised *M. tuberculosis* and mannose receptors, and scavenger receptors for the uptake of nonopsonised *M. tuberculosis*. 4) Recognition receptors and Toll-like receptors (TLRs) are expressed not only at the cell surface but also in phagosomes; therefore, immune activation may occur with or without phagocytosis. NOD: nucleotide-binding oligomerisation domain protein; NLRP3: NOD, LRR and pyrin domain containing 3; DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin.

do not induce strong inflammatory responses. Their ability to produce anti-microbial chemicals such as nitric oxide (NO) and reactive oxygen intermediates (ROI) is blunted [13].

Innate immunity

The recognition of *M. tuberculosis* components by multiple pattern-recognition receptors on alveolar macrophages initiates innate immunity. Of the Toll-like receptors (TLRs), TLR2 has the largest number of identified mycobacterial agonists, including lipoproteins (as many as 99 of them), phosphatidylinositol mannans and lipomannan [14]. In addition, TLR9 senses mycobacterial DNA and contributes to the production of cytokines by macrophages and dendritic cells [15]. Additional recognition of *M. tuberculosis* is mediated by specific members of the C-type lectin receptor family, including the lectin dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN) [16] and the mannose receptor [17]. Of the cytosolic pattern-recognition receptors, nucleotide-binding oligomerisation domain protein 2 (NOD2) [18] and NOD, NOD-like receptors (NLRs) and pyrin domain containing 3 (NLRP3) [19] recognise the peptidoglycan subunit *N*-glycolyl muramyl dipeptide and one or more extraembryonic, spermatogenesis, homeobox 1 homologue (ESX1)-secreted substrates (such as the 6-kDa early secretory antigenic target (ESAT6)), respectively. Stimulation of these pattern recognition receptors, individually or collectively, induces the expression of pro-inflammatory cytokines.

Circulating blood monocytes are recruited through chemokine signals produced by infected alveolar macrophages, and migrate rapidly across the blood vessels to the site of infection. Within the tissue, they differentiate into macrophages with the ability to ingest and kill the bacteria. Interaction between macrophages and T-cells (and in particular, the activation of macrophages by interferon (IFN)-γ secreted by T-cells) is considered central in the elimination of *M. tuberculosis* [20]. However, as these cells are recruited, they become infected by the expanding population of mycobacteria and establish early granulomas. In other infectious diseases, the recruitment of phagocytic cells restricts and even eliminates invading pathogens, whereas the recruitment of phagocytes to sites of mycobacterial infection actually benefits the pathogen during the early stages of infection, by providing additional cellular niches for bacterial population expansion [21].

Adaptive immunity

A peculiar characteristic of the adaptive immune responses to *M. tuberculosis* infection is the long delay in onset. Measurable adaptive immune responses emerge in humans approximately 42 days after *M. tuberculosis* exposure [22] and a similar delay is observed with hepatitis C virus infection [23]. In contrast, immune responses can be activated within ~20 hours postinfection with the influenza virus [24]. It is currently unclear why this step is so prolonged, although there is evidence that *M. tuberculosis* infection of myeloid dendritic cells inhibits their migration in response to ligands for CC chemokine receptor 7 (CCR7) [25]. After the onset of adaptive immunity with the accumulation of effector CD4+ and CD8+ T-cells in the lungs, the growth of the bacterial population is arrested and most patients become asymptomatic, do not shed bacteria and are considered to have latent TB infection (LTBI). The delayed adaptive immune response could be significant in establishing latency by giving the bacteria time to establish sufficient numbers to evade complete elimination. Multiple mechanisms probably contribute to the limited ability of adaptive immune responses to kill *M. tuberculosis*, some of which are well characterised and include: 1) impaired major histocompatibility complex (MHC) class II-mediated antigen presentation [26]; 2) induction of the anti-inflammatory mediator lipoxin A4 [27]; 3) restriction by regulatory T-cells [28]; 4) down-regulation of bacterial antigen gene expression and, therefore, failure to induce antigen-specific CD4+ T-cells [29]; and 5) resistance to the macrophage-activating effects of IFN-γ [30]. Moreover, a recent study in non-human primates revealed that *M. tuberculosis* also accumulates mutations during latency [31]. Taken together, these data provide convincing evidence that LTBI is not simply a state of bacterial stasis, but a state of dynamic bacterial and immunological equilibrium. Thus, inflammation is a double-edged sword in the host

response to *M. tuberculosis*. On the one hand, it is required for the initial control of infection to bring the bacteria into homeostasis with the human host. Failure to mount an adequate inflammatory response leads to progressive disease upon infection in both animal models and genetically susceptible humans. On the other hand, the bacteria exploit the host inflammatory response to spread to susceptible individuals and to fill as completely as possible their ecological niche.

Mechanisms of immune evasion

Considerable evidence indicates that *M. tuberculosis* and other pathogenic mycobacteria of the *M. tuberculosis* complex have evolved multiple mechanisms to manipulate their cellular niches for their own advantage (fig. 2). First, pathogenic mycobacteria modulate the trafficking and

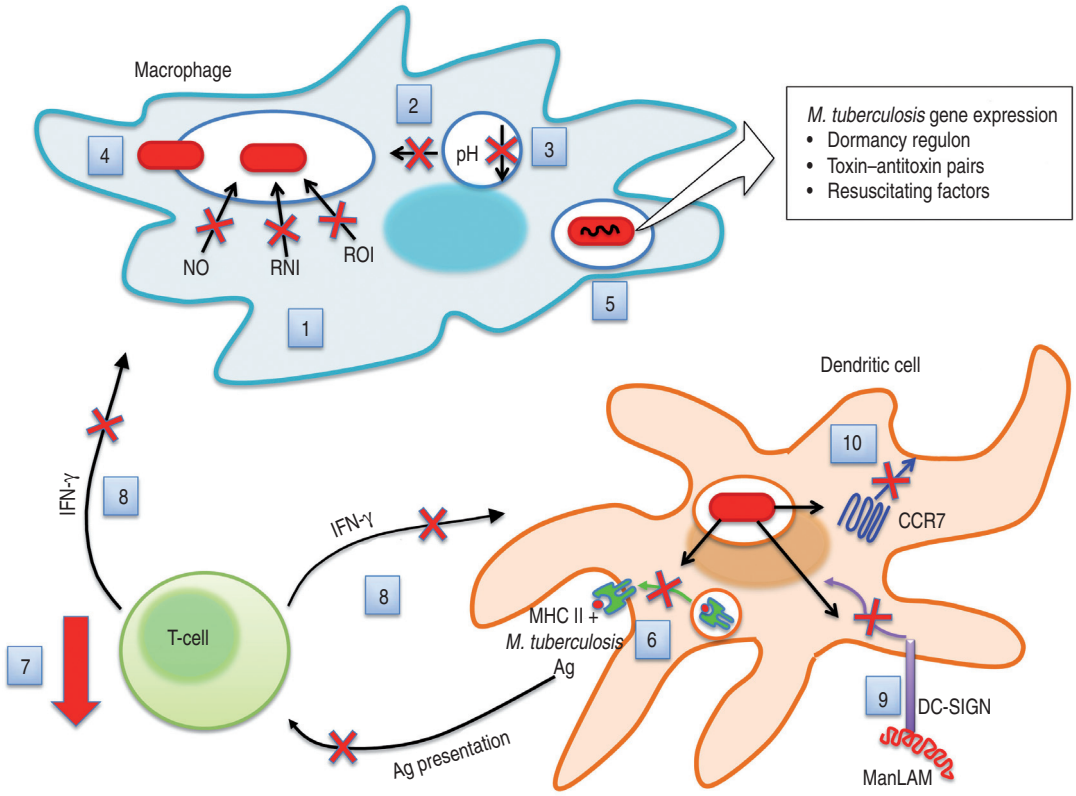

Figure 2. Mechanisms of immune evasion by *Mycobacterium tuberculosis*. 1) *M. tuberculosis* makes the macrophages a ''sanctuary'' and enables the bacteria to resist killing by nitric oxide (NO), reactive nitric oxide intermediates (RNI) and reactive oxygen intermediates (ROI); 2) by blocking the phagosome–lysosome fusion; and 3) by inhibiting lysosome acidification. 4) Virulent strains of *M. tuberculosis* can translocate from the phagosome to the cytosol, bacille Calmette–Guérin cannot. This capacity is linked to the ESX-1 coding region (within region of deletion (RD)1). 5) *M. tuberculosis* has evolved specific mechanisms to adopt and recover from a state of latency and that latency is not merely the suppressive effect of the host immune response on bacterial replication. 6) *M. tuberculosis* disrupts antigen (Ag) presentation through the downregulation of major histocompatibility complex (MHC) class II and 7) consequently causes a decrease in the antigen-specific CD4 T-cell population. 8) Insufficient interferon (IFN)-γ is essential for both macrophage and dendritic cell (DC) maturation and development of an efficient cellular immune response against *M. tuberculosis* infection. 9) Ligation of dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (DC-SIGN) by *M. tuberculosis* virulence factor mannose-capped cell wall component lipoarabinomannan (ManLAM) reduces Toll-like receptor 4-triggered DC maturation and enhances the production of interleukin-10, thus resulting in inhibition of DC maturation. 10) Moreover, the migration of infected DCs to lymph nodes in response to CC chemokine receptor (CCR)7 ligands is impaired by *M. tuberculosis* induced downmodulation of CCR7.

maturation of the phagosomes in which they reside [32], allowing them to evade lysosomal mechanisms of restriction, killing and degradation. Secondly, mycobacteria use several virulence mechanisms to optimise their spread from cell to cell. For example, the ESX1 type VII secretion system, the absence of which attenuates the strain of *M. bovis* used in the BCG vaccine [33], promotes the necrotic death of infected cells and the recruitment of macrophages. This allows the intracellular bacteria to be released from the cell for uptake by the freshly recruited adjacent phagocytes, resulting in subsequent intracellular growth and bacterial population expansion [21]. Thirdly, *M. tuberculosis* possesses multiple mechanisms for inhibiting host cell apoptosis [34]; among other benefits to the bacteria, such inhibition allows for the prolonged survival of infected cells and for a larger number of bacteria to accumulate in a given cell before they are released by cell death [25].

Strong evidence exists that the mycobacteria are also active contributors to the immunological equilibrium state in LTBI. First, a well-characterised bacterial regulon that is controlled by DosR–DosS, a two-component signal transduction system in mycobacteria, is induced by several stimuli thought to prevail during LTBI, including local hypoxia [35], NO [36] and carbon monoxide [37]. This dormancy regulon controls the expression of genes that allow the bacteria to use alternative energy sources, especially lipids, and genes encoding factors that are selectively recognised by T-cells from humans with LTBI (but not active TB infection) [38]. The expression of this gene network implies that *M. tuberculosis* has evolved specific mechanisms to adopt a state of latency, and that latency is not merely the suppressive effect of the host immune response on bacterial replication. In addition, *M. tuberculosis* encodes five proteins that resemble the well-characterised *Micrococcus luteus* resuscitation-promoting factor (Rpf), which is a secreted protein that has the ability to "resuscitate" bacteria from a nutrient-starved dormant state [39]. Deletion of one or more of the *M. tuberculosis rpf* genes generates bacteria that have an impaired recovery from dormancy, indicating that these genes may participate in the progression from latency to reactivation [40]. Finally, *M. tuberculosis* encodes 88 toxin–antitoxin gene pairs, the expression balance of which regulates multiple phenomena, including whether the bacteria replicate or remain static [41]. Thus, *M. tuberculosis* possesses at least three systems (the dormancy regulon, resuscitation promoting factors and toxin–antitoxin gene pairs) that regulate its metabolic and growth state.

Mechanisms of TB reactivation

Most cases of TB in adults are attributable to reactivation that can occur decades after the initial infection [42]. Reactivation of TB is widely attributed to weakened immunity, although only a minority of cases can be linked to well-characterised defects in immunity. In humans, only two mechanisms have been identified that explain reactivation of TB. 1) The quantitative and qualitative CD4+ T-cell defects that occur in people infected with HIV [43]; although the precise mechanisms that these cells use to establish and maintain immune control in the latent state of the disease remain to be identified. 2) The therapeutic neutralisation of tumour necrosis factor (TNF) [44], that results in decreased macrophage-mediated anti-mycobacterial activity and the subsequent death of macrophages [45]; the induction of a higher frequency of regulatory T-cells [46]; and the depletion of a subset of CD45RA+ effector memory CD8+ T-cells that contain granulysin [47].

As noted previously, *M. tuberculosis* has specific programmes for initiating a state of dormancy in response to certain environmental signals (some of which are imposed by adaptive immune responses) and this state manifests as clinical latency. In turn, *M. tuberculosis* also has specific programmes for recovering from dormancy, suggesting that the bacteria may assume a primary role in some cases of reactivation TB that are not explained by immune defects or deficiencies.

The development of efficacious vaccines against TB presents unique challenges that demand a better understanding of protective and pathological immune responses in TB. Clear correlates of protective immunity have not yet been identified, especially in humans, making surrogate end-points inadequate for evaluating TB vaccine efficacy.

New diagnostic tests for TB

TB still remains a serious public health threat. More than 9 million new cases are reported annually, and the incidence rate is falling at less than 1% per year [48]. The current TB diagnostic pipeline is vastly better than the portfolio available 10 years ago when smear microscopy, a 100-year old test, was the only option for most resource-limited settings (fig. 3) [49]. Despite being the cornerstone of TB diagnosis in low-resource settings, smear microscopy has only modest sensitivity for TB disease and particularly low sensitivity in patients with advanced HIV disease (fig. 4). In settings with a high prevalence of HIV infection, current tools and strategies for diagnosis of TB are inadequate. Additionally, poor access to TB diagnostics continues to be a major challenge contributing to under-diagnosis of disease, which leads to individual morbidity and mortality and to continued transmission and delayed diagnosis of drug resistance, leading to acquisition of additional resistance and to morbidity and transmission. The lack of accurate and rapid diagnostics remains a major obstacle in the progression of the detection rate for new sputum smear-positive and smear-negative pulmonary cases of TB, childhood TB and extrapulmonary (EP)TB. This is of importance for the control of both sensitive and drug-resistant (DR)-TB at national and global level [50]. Development of resistance to antituberculotic drugs, and especially multidrug-resistant (MDR)-TB (defined as TB resistant to isoniazid and rifampicin) and extensively drug-resistant (XDR)-TB (defined as MDR-TB resistant to second-line injectables and fluoroquinolones) are threats to the elimination of TB worldwide. The ability to rapidly and accurately detect *M. tuberculosis* in clinical specimens, as well as drug resistance, is essential for the appropriate treatment of TB patients and the prevention of spread of drug-resistant strains.

Point-of-care tests

There is a great need for rapid point-of-care tests that can be readily used at all levels of the health system and in the community [51]. These techniques are often unsatisfactory and unavailable at patients' first point of contact with the health system. Notable advances in TB diagnostic technologies have been made in the past several years, and the potential exists for translating these developments into meaningful improvements in global TB clinical care and control. Unfortunately no real point-of-care tests will be commercially available in the next few years.

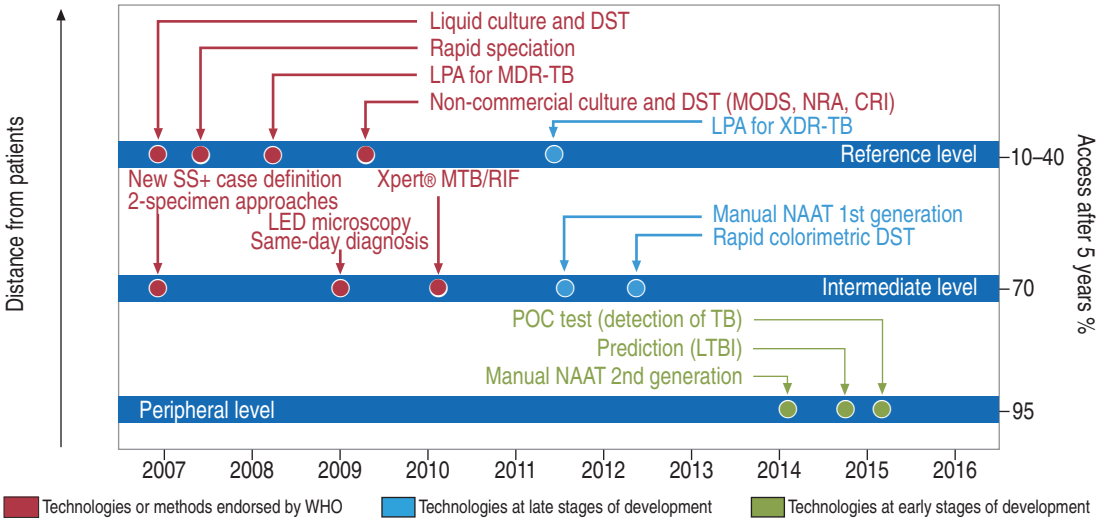

Figure 3. The pipeline for new diagnostics. Xpert® MTB/RIF is manufactured by Cepheid (Sunnyvale, CA, USA). DST: drug-susceptibility test; LPA: line probe assay; MDR: multidrug-resistant; TB: tuberculosis; MODS: microscopy observed drug susceptibility; NRA: nitrate reductase assay; CRI: colorimetric redox indicator assay; XDR: extensively drug-resistant; SS+: sputum smear positive; LED: light-emitting diode; NAAT: nucleic acid amplification techniques; POC: point-of-care; LTBI: latent TB infection; WHO: World Health Organization. Reproduced and modified from [48] with permission from the publisher.

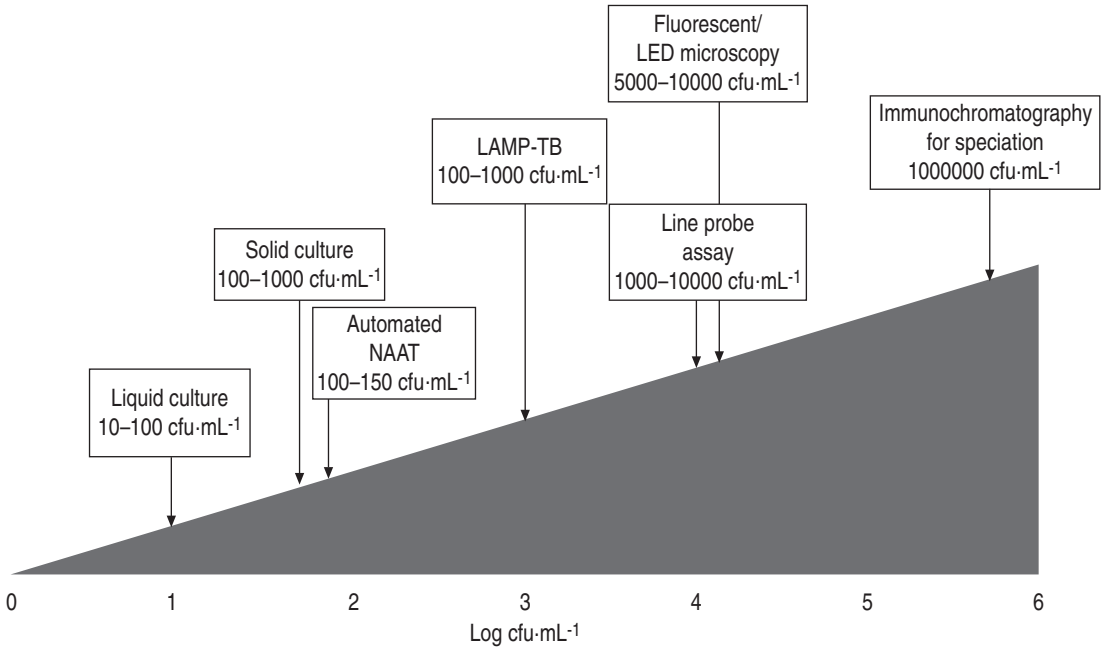

Figure 4. Sensitivity of the current diagnostics: liquid cultures performed by automated systems are the most sensitive tool available today, automated nucleic acid amplification techniques (NAAT) (Xpert® MTB/RIF; Cepheid, Sunnyvale, CA, USA) shows a sensitivity comparable to solid cultures. LAMP: loop-mediated amplification; TB: tuberculosis; LED: light-emitting diode.

Microscopy and culture

Smear microscopy and solid culture have been the cornerstone of TB laboratory diagnosis for decades. Some new technologies have been endorsed by the World Health Organization (WHO), such as implementation of automated liquid cultures, fluorescence microscopy and light-emitting diode (LED) fluorescence microscopy [52, 53]. The WHO is also recommending new policies for sputum collection and the number of samples to be examined by smear microscopy for the definition of a TB case. Culture of *M. tuberculosis* in clinical specimens is substantially more sensitive than smear microscopy. Culture can be performed using solid media, such as Lowenstein–Jensen, or liquid media, such as the ones used in commercially available automated systems. Until the recent advent of molecular tests for drug resistance, isolation of *M. tuberculosis* by culture was a prerequisite for subsequent phenotypic drug-susceptibility testing (DST). The Achilles' heel of culture is the time it takes to acquire results (10–14 days for liquid culture and 3–4 weeks for solid culture), which is a consequence of the long doubling time of *M. tuberculosis*. Currently available culture methods are technically demanding, require implementation of biosafety practices and equipment to prevent inadvertent infection of laboratory personnel, and have relatively high per test prices. Automated liquid culture systems are now the gold standard for the diagnosis of TB; they are substantially faster and have a 10% greater yield than solid media. In 2007, these systems were recommended by WHO to be used in combination with antigen-based species confirmation for diagnosis and DST in low-income and middle-income countries. However, such systems are expensive and prone to contamination. Alternative inexpensive noncommercial culture and DST methods were endorsed by WHO in 2009 for use as an interim solution in resource-constrained settings [53]. These alternatives include microscopy observed drug susceptibility (MODS) and the nitrate reductase assay [54].

Molecular tests for diagnosis of DR-TB

To enhance the capacity for rapid diagnosis of MDR-TB, in 2008, WHO approved the use of line probe assays (LPAs) for the rapid molecular detection of drug resistance in smear-positive

specimens or culture isolates. These molecular assays reduce the time to diagnosis of MDR- and XDR-TB from weeks or months to a matter of days. However, it has yet to be shown whether the use of such assays improves patient outcomes. Nucleic acid amplification techniques (NAATs) are the most promising development in TB diagnostics. These tests have been shown to have high specificity, but limited and variable sensitivity, especially for sputum smear-negative disease. Simplified versions of these assays with higher sensitivity are being developed. A simplified manual NAAT that uses loop-mediated isothermal amplification with a simple visual colorimetric readout is being assessed in peripheral laboratory facilities in resource-constrained settings [55]. A new rapid test that overcomes many of the current operational difficulties was endorsed by WHO in December 2010, the Xpert® MTB/RIF assay is a sensitive and specific fully automated real-time nucleic acid amplification technology run on the multi-disease platform GeneXpert® (Cepheid, Sunnyvale, CA, USA). The Xpert® MTB/RIF assay that simultaneously detects *M. tuberculosis* and rifampicin resistance-conferring mutations, in a closed system, in less than 2 hours, directly from sputum samples has been developed for use outside reference laboratory centres. This system uses a series of molecular probes and real-time PCR technology to detect *M. tuberculosis* and the *rpoB* rifampicin resistance mutation [56–60]. The Xpert® MTB/RIF assay has proven a valid tool also for the diagnosis of EPTB [61].

LPA technology remains a valid tool for fast detection of the MDR phenotype in smear-positive samples [62–64]. The newest generation of LPAs for detection of resistance to rifampicin and isoniazid shows an increased sensitivity also in paucibacillary specimens. A second-line drug LPA test is commercially available; the test targets the main mutations causing resistance to injectables, fluoroquinolones and ethambutol. The test shows a high positive predictive value (PPV) for injectables and fluoroquinolones; however, the negative predictive value is low and the test cannot be used for excluding XDR-TB [63, 64].

For ethambutol, the correlation of the molecular test targeting the *embB306* mutation with phenotypic tests performed *in vitro* is low, this could also be due to the suboptimal performance of *in vitro* testing for ethambutol resistance performed by Bactec™ MGIT™ (BD Bioscience, Erebodegem, Belgium) [64].

IFN-γ release assays

For the past century, the tuberculin skin test (TST) using purified protein derivative has been the only screen available for the diagnosis of LTBI [51]. Together with chest radiographs, TST is used as an adjunct to smear microscopy (and culture, if available) in some settings; however, the former have poor sensitivity and specificity for active TB, and the latter are often not available at the point of primary patient care. In detecting LTBI, the TST's major failing is its inability to reliably distinguish individuals infected with *M. tuberculosis* from individuals sensitised to other mycobacteria, including BCG [51]. A decade ago IFN-γ release assays (IGRAs) were developed whereby IFN-γ titres were measured after *in vitro* stimulation of peripheral blood mononuclear cells with antigens such as ESAT-6 and the 10-kDa culture filtrate antigen (CFP-10) (immunodominant antigens expressed by members of the *M. tuberculosis* complex) [4, 65]. The IGRAs are used principally for detection of *M. tuberculosis* infection. Use of these tests for the diagnosis of active disease is based on the presumption that one must have TB infection in order to have TB disease. The greater problem in diagnosing active TB is their poor specificity for disease, because these tests cannot distinguish an immune response to reactivated TB from a response to TB infection that remains latent. Nevertheless, IGRAs have now become the gold standard in industrialised countries for identifying individuals whose immune system has previously encountered *M. tuberculosis* and have been extensively tested in many clinical situations and in individuals infected with HIV. The assessment of these test results for detection of LTBI has been difficult because of the absence of a gold standard for TB latency [51]. A meta-analysis of these studies showed that IGRAs are at least as sensitive as and more specific than TST [4]. Longitudinal studies have shown that the predictive value of IGRAs for reactivation of TB in immunosuppressed individuals is better than that provided by the TST in individuals vaccinated with BCG. High levels of IFN-γ release are detected by these assays in about

70–90% of individuals with active disease and these levels decrease after treatment is completed, although such reductions are not consistently recorded [4, 66].

Beyond new technology

Successful implementation of new tools will depend on more than technological innovation. At the research level, rigorous implementation of well-designed, bias-minimised studies and complete and accurate reporting are essential for appropriate decision making by the healthcare community charged with implementing tests for individual patient evaluation or recommending tests for TB programme use.

Molecular approaches for diagnosis of drug resistance will certainly be implemented in the future and tests designed using information made available by the large scale use of new-generation sequencing will allow the design of more specific and sensitive assays.

New programmatic approaches, including revised clinical algorithms for TB diagnosis, may be needed to maximise the impact of new tools. For example, should rapid molecular tests for drug resistance be performed for all persons with suspected TB during the initial evaluation, be reserved for use in the initial evaluation only of persons with suspected TB and risk factors for drug resistance, or be used in some other place in a diagnostic algorithm? In populations with a high prevalence of HIV infection, should urine-based antigen detection tests be used solely for evaluation of symptomatic persons with suspected TB, or should they also play a role in routine screening of HIV-infected persons? To date, most TB diagnostic test development has focused on maximising sensitivity and specificity to rule in or confirm a TB diagnosis. However, a test with an exceedingly high negative predictive value might have use in ruling out TB and, thereby, allowing efficient triage of patients and resources; such a test would require careful assessment to determine its optimal use in clinical algorithms.

Laboratory capacity needs to be strengthened, especially in resource-limited settings [67]. Although some aspects of laboratory strengthening will vary according to the characteristics of the new tests, there are, nevertheless, general unmet needs, including those for training at technologist and management levels, retention of trained personnel, enhancement of quality-assurance systems, enhancement of result-reporting mechanisms, and reliable mechanisms for instrument maintenance and supply procurement.

Funding estimates aside, it is clear that important advances in TB diagnosis have recently been made, and potentially useful new tools are emerging; continued and augmented investment will be required to successfully implement the most promising of these tools in the settings where they are most needed and to maintain a robust pipeline that will ultimately yield the tools that revolutionise TB diagnosis.

Conclusions

What's new in TB diagnostics? There are a lot of new developments, but not enough. The future is brighter as several promising new tools enter the demonstration and late evaluation stages, but there is a great need for improvement and important barriers still remain in translating technical advances into meaningful and sustainable improvements in individual and public health in settings hardest hit by TB.

Statement of Interest

None declared.

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Chapter 2

Epidemiology of TB

Lia D'Ambrosio[*], *Antonio Spanevello*[*,#] *and Rosella Centis*[*]

SUMMARY: Although *Mycobacterium tuberculosis* was discovered over a century ago, and different generations of effective tuberculosis (TB) drugs and regimens have been discovered and are widely used within TB control programmes, TB still represents a first-class health priority at the global level in terms of death and disability.

Several determinants, in addition to drugs, contributed to modify TB trends in different parts of the world, leading to the overall decline we have observed in the last few years. Among them, multidrug-resistant (MDR) and extensively drug-resistant (XDR)-TB, the HIV pandemic, and other social determinants have influenced the epidemiological features of the disease.

In this chapter, we will discuss how TB transmission occurs in humans (through a separate description of exposure, infection, disease and death) and how to measure its core steps by means of *ad hoc* specific indicators.

KEYWORDS: Epidemiology, extensively drug-resistant tuberculosis, multidrug-resistant tuberculosis, tuberculosis

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Tuberculosis (TB) is still a leading cause of death and disability over a century after the discovery of *Mycobacterium tuberculosis* by Robert Koch, and several decades after effective anti-TB drugs were discovered and have become widely used [1, 2]. Several causes combined to reduce the incidence of TB over time, primarily the improvement of socioeconomic conditions in Europe (fig. 1).

Many factors influence the global epidemiology of TB, and explain why, after much effort, the global TB epidemic is finally reversing [3, 4]. Several core issues influence the epidemiological features of the disease, including multidrug-resistant (MDR) and extensively drug-resistant (XDR)-TB, the HIV pandemic, and other social determinants [3–5].

The present chapter will discuss the global epidemiology of TB, beginning with the dynamics and interactions between *M. tuberculosis* and human beings in a specific environment. Furthermore, it will qualitatively and quantitatively evaluate the covariates (*e.g.* the administration of anti-TB drugs) that could interfere with the outcomes of the natural history of this interaction (*i.e.* exposure, infection, disease and death) [6].

The chapter will first discuss how TB transmission occurs and how we can model it to describe exposure, infection, disease and death. It will then show how we can measure these core steps with specific indicators.

TB transmission and its steps

M. tuberculosis spreads from one person to another essentially *via* airborne transmission, having a large reservoir within the human population [2, 6, 7]. All the alternative routes of transmission are rarely reported (skin lesions, transplacental, *etc.*) and are not epidemiologically relevant. *Mycobacterium bovis*-sustained infection through non-pasteurised or -boiled milk from cows with TB mastitis, which was common in the past, is currently rare [6]. Therefore, control and elimination of TB are based on airborne transmission through the epidemiological model schematised in figure 2 (more on control and elimination can be found in Chapter 18 [8]). In fact, the primary objective is to reduce and to eliminate those determinants promoting the progression from one step to the next in this model [6, 9, 10].

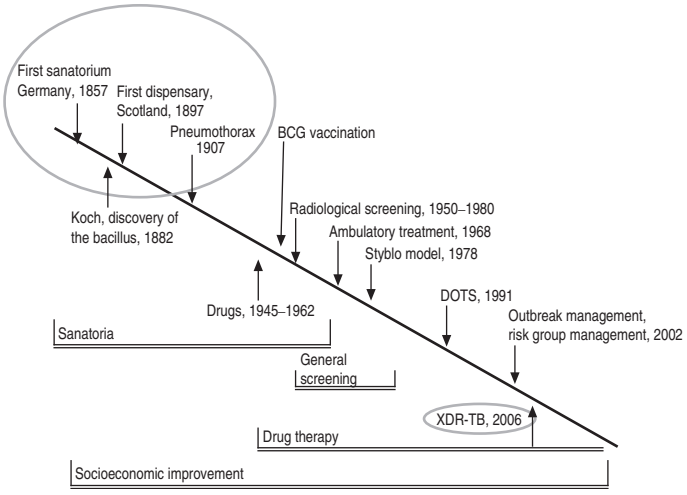

Figure 1. History of tuberculosis (TB) control interventions shown over the declining curve of TB incidence in a generic country. BCG: bacille Calmette–Guérin; DOTS: directly observed treatment, short course; XDR: extensively drug-resistant. Reproduced from [2] with permission from the publisher.

Exposure

The main factors determining the risk of *M. tuberculosis* exposure are the number of TB infectious cases (*e.g.* those with a positive sputum smear), the duration of their infectiousness and the patterns of their social mixing. The more cases with an infectious sputum smear-positive form of TB, the greater the number of mycobacteria present in the environment. Similarly, the longer these cases remain infectious, the longer there are mycobacteria in the environment [6]. However, people need to share the same environment with the TB infectious cases to be exposed, which depends on social behaviours and opportunities for contact [11]. Higher exposures are reported in environments with high population density, such as large households, confined spaces (*e.g.* prisons, hospitals and other congregate settings) and urban areas [12, 13]. In addition, households represent a priviledged site where transmission occurs. Exposure could also be related to an individual's sex in those countries where females have a confined social life and, consequently, lower exposure to TB. A converse example would be settings in which only males are present (*e.g.* overcrowded prison camps where soldiers are confined or mining villages) [6].

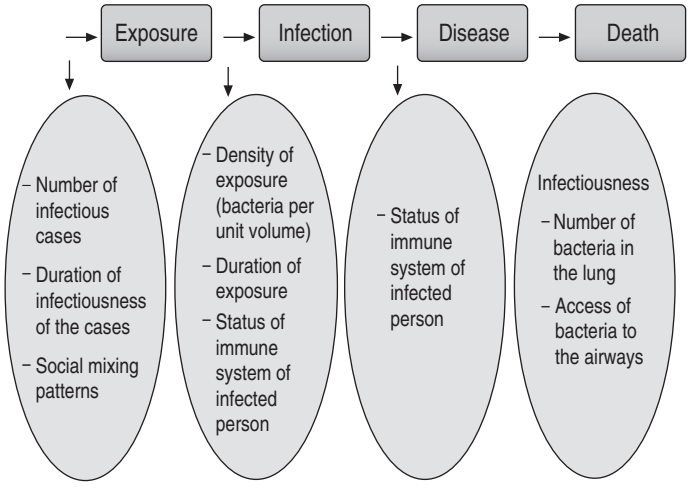
Exposure
Infection
Disease
Death
– Number of infectious cases
– Duration of infectiousness of the cases
– Social mixing patterns
– Density of exposure (bacteria per unit volume)
– Duration of exposure
– Status of immune system of infected person
– Status of immune system of infected person
Infectiousness
– Number of bacteria in the lung
– Access of bacteria to the airways

Figure 2. Simplified epidemiological model describing the determinants of exposure, infection, disease and death.

The role played by infection control in reducing the risk of exposure and the probability of infection will be described the section entitled Disease [14, 15].

Infection

The determinants in the transition from exposure to infection are the density and duration of exposure and the status of the immune system of the person exposed [6, 12]. The density of exposure is measured by the number of infectious droplet nuclei per air volume. Talking, coughing, sneezing or singing produce infectious droplets. The longer the exposure, the higher the chances of inhaling infectious droplets, which are able to stay buoyant in the air for up to several hours, and become infected. Air circulation and ventilation can decrease the load of buoyant infectious droplets. Those droplets that are small enough to enter deep into the lungs and adhere to the alveolar cells can be killed by macrophages or establish a local infection. The macrophage response may be weakened by a number of genetic, environmental and medical factors (*e.g.* HIV co-infection) [6, 12, 16, 17]. Factors associated with *M. tuberculosis* itself may also favour infection (and disease), such as cell number (infecting dose) and strain virulence [6, 12, 16, 17].

Disease

In the event of infection, the status of the immune response also determines the probability of developing TB disease [18]. When affected by TB, a person is contagious proportionally to the number of mycobacteria in the lung cavities and to their chance of accessing the airways (either lower or upper airways) in order to be included into droplet nuclei. 2-cm lung cavities can contain up to 100 million mycobacteria. There is therefore a cycle from infectious TB disease to exposure (fig. 3). The risk of developing TB disease, given infection, is shown in figure 4, being $<10\%$ of lifetime in immunocompetent and 30% or more in immunocompromised individuals [6, 12]. As the risk is higher in the first 2 years after infection, a rationale exists for targeting recent converters for treatment of latent TB infection (LTBI) [19].

Based on the evidence raised by K. Styblo through his annual risk of TB infection model, transmission of TB follows specific patterns as described in figure 4 [2, 20]. Figure 4a shows what happens under natural history conditions. One infectious source infects, on average, 20 individuals (10 per year in 2 years). 10% of them develop TB disease and 50% become sputum smear positive (*i.e.* infectious). In the presence of HIV (fig. 4b), the time available for infection is reduced, the breakdown rate increased up to 50% and the probability of becoming sputum smear positive is 40%. In this hypothetical scenario, each source of infection produces two sources. In the presence of unfavorable social determinants, even if interventions are available (DOTS (directly observed treatment, short course) and the Stop TB Strategy, see later), the breakdown rate increases up to 20%, still producing two infectious cases per source (fig. 4c). Treatment (fig. 4d), by reducing the time of infectiousness, reduces the number of infectious cases produced by each source to 0.5. Figure 4e shows the best possible scenario, when effective DOTS (early diagnosis and treatment) is available. Under these circumstances, one source produces 0.25 infectious cases. Figure 4f demonstrates the danger of irregular treatment. Through increasing duration of the infectious period, each source produces 1.5 infectious cases. This scenario is alarming, showing that a programme with a low success rate progressively deteriorates the epidemiology by increasing the number of TB cases and by producing drug-resistant (DR) and MDR-TB cases. Figure 4g shows that, under high HIV prevalence, even a good DOTS programme cannot improve the situation, the answer being introduction of antiretroviral treatment (ART).

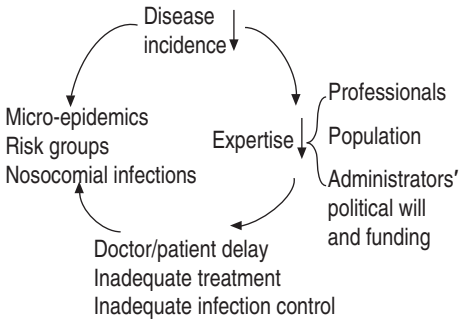

Figure 3. Problems and challenges in tuberculosis control and elimination. Reproduced from [9] with permission from the publisher.

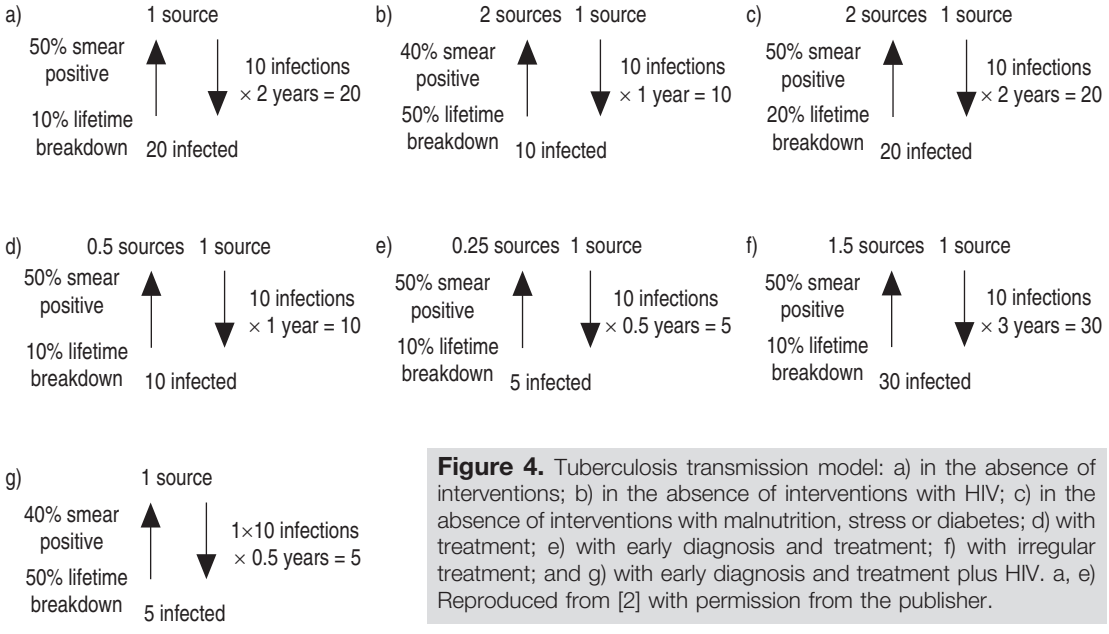

Figure 4. Tuberculosis transmission model: a) in the absence of interventions; b) in the absence of interventions with HIV; c) in the absence of interventions with malnutrition, stress or diabetes; d) with treatment; e) with early diagnosis and treatment; f) with irregular treatment; and g) with early diagnosis and treatment plus HIV. a, e) Reproduced from [2] with permission from the publisher.

Descriptive epidemiology

The global burden of TB is measured using standard epidemiological indicators (incidence, prevalence, mortality and case fatality). Estimates of these indicators are collected every year by the World Health Organization (WHO), which gathers together information obtained from surveillance systems (notification systems and mortality registers), special epidemiological studies (surveys of TB prevalence and in-depth analyses of surveillance data) and expert opinion [3].

Epidemiological indicators

TB incidence

TB incidence is the number of new cases of TB occurring within a specific time period (usually 1 year) in a defined cohort. It is usually presented as a rate per 100,000 inhabitants and describes the probability of developing TB in a specific time period.

TB prevalence

TB prevalence is the number of cases of TB in a defined population at a specific point in time. It is presented as an absolute or a relative (usually per 100,000 inhabitants) frequency and can be considered the product of the incidence of TB and the duration of the disease. This indicator assesses the global, national, regional and local burden of TB.

TB mortality

TB mortality is the number of deaths from TB occurring within a specific time period (usually 1 year) in a cohort of individuals with TB disease. It is often presented as a rate per 100,000 inhabitants and describes the probability of dying from TB in a specific time period.

TB case fatality

TB case fatality is the number of deaths from TB occurring within a specific time period (usually 1 year) in a defined cohort of individuals with TB. It is presented as a percentage.

Incidence of sputum smear-positive pulmonary TB cases

The incidence of sputum smear-positive pulmonary TB cases is the number of new sputum smear-positive cases of pulmonary TB occurring within a specific time period (usually 1 year) in a defined cohort. It is usually presented as a rate per 100,000 population. It identifies the most contagious TB cohort, *i.e.* the most important source of infection.

Incidence of sputum culture-positive pulmonary TB cases

The incidence of sputum culture-positive pulmonary TB cases is the number of new sputum culture-positive cases of pulmonary TB occurring within a specific time period (usually 1 year) in a defined cohort. It is usually presented as a rate per 100,000.

Surveillance

TB (and MDR-TB) surveillance is an epidemiological tool designed to monitor the spread of the disease to establish patterns of progression. The main role of TB and MDR-TB surveillance is to observe, predict and minimise the harm caused by outbreaks and epidemics/pandemics, as well as to decipher the key risk factors involved.

Surveillance is an essential part of any effective national TB programme (NTP). Thus, the staff responsible for managing the programme at all levels should have a clear understanding of surveillance and its importance [21]. Indeed, public health surveillance is the ongoing, systematic collection, analysis and interpretation of outcome-specific data, followed by the timely dissemination of those data to other individuals who share responsibility for the prevention and control of the disease [22, 23].

TB surveillance has local, national and international functions [23]. At a local level, it is relevant to ensure both individual management (*i.e.* that appropriate treatment is offered to the individual patient and contact tracing is ensured) and population surveillance (*e.g.* recognition of outbreaks and evaluation of local epidemiology). At national and international levels, more emphasis is given to population-based activities: monitoring the epidemiology of TB (including trends over time and variations in the incidence in specific subgroups), the success of the national treatment programme, and the effectiveness of specific control and prevention measures.

Several principles should guide TB surveillance: 1) information on cases should be collected at the lowest possible level; 2) information sources should be comprehensive to increase the sensitivity of the system (clinicians and laboratories, compulsorily; pharmacies, social security organisations and other sources, possibly); 3) a standard case definition at a national level should be used; 4) data on individual cases should be collected at national levels to allow detailed analysis of reported cases, elimination of duplicate reports and establish nominal links with registers of other diseases; 5) confidentiality must be ensured; and 6) a minimum set of essential variables should be included in the notification form [23, 24].

In 1995, a new global project on TB surveillance and programme monitoring was established by WHO to describe the magnitude of the global TB epidemic and to assess the status of global control measures. Case data collection forms and NTP forms were developed to assess performance at national levels and are sent annually to all WHO member states and some other countries and territories.

Because drug resistance is often due to inadequate treatment, indicators of successful treatment may be useful for monitoring the efficiency of an NTP. In 1994, WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD) developed a set of guidelines to assist NTPs to establish policies for resistance surveillance [25]. In 1995, WHO and the IUATLD implemented a global project on anti-TB drug resistance surveillance designed to: 1) collect data on the extent and severity of anti-TB drug resistance; and 2) monitor, in a standardised manner, the global prevalence of drug resistance at a national level. The strategy to achieve these objectives

consisted of the implementation of standardised epidemiological and statistical methodologies, and the guidance of a network of supranational reference laboratories to assure quality control.

Based on this information framework and on data collected using the WHO Monitoring and Evaluation tool [26], the annual WHO TB Report [3] and periodic reports on MDR-TB [27] are developed, allowing a precise picture of the global TB epidemiological situation.

Furthermore, surveillance systems should be equipped to report treatment outcomes for at least 24 months following treatment initiation to capture the outcomes of MDR- and XDR-TB cases. Although European countries are taking action to improve their surveillance systems, most of the information presently available on XDR-TB is based on *ad hoc* studies.

Epidemiology of TB

WHO estimates that, in 2010, there were 8.8 million new TB cases worldwide (3.2 million among females) and 1.45 million deaths (half a million among females). In 2010, there were 9.7 million orphans whose parents died of TB. Of all cases, 1.1 million (13%) occurred among people living with HIV, of which 350,000 were fatal. About 650,000 cases of MDR-TB were present in 2010, while XDR-TB has been reported officially by 69 countries [3].

The burden of TB is now slowly decreasing globally, after years of continuous growth, particularly in Eastern Europe and in the WHO Africa Region. However, the rate of decline is still too slow to reach all the epidemiological impact targets discussed previously. Among the most serious challenges, implementation of TB/HIV collaborative activities and management of DR-TB need to be mentioned. To attain effective TB control, there is an urgent need to ensure that all components of the Stop TB Strategy are scaled up [28], with special attention to improved access for the poor and removal of those TB risk factors and social determinants (overcrowded living conditions, alcoholism, malnutrition, diabetes and smoking) on top of advocating the necessary new diagnostic and treatment measures [5, 12, 29].

Drug-resistant TB

MDR- and XDR-TB are strong indicators of TB control programme failures. MDR- and XDR-TB occur for several reasons, including the following [3, 30–33]. 1) Care providers may prescribe insufficient drug regimens for patients (*e.g.* inadequate doses or numbers of drugs, incorrect drug choice or inadequate durations of treatment). 2) Patients may not adhere to an appropriate regimen (*e.g.* interrupting or discontinuing treatment). 3) Drugs used may be of poor or substandard quality; this is particularly frequent when fixed-dose combinations are used. All of these factors may contribute to the development of acquired drug resistance in patients being treated for pan-susceptible TB. In addition, when MDR- and XDR-TB are left undetected or untreated, they may be transmitted directly from one individual to another, resulting in primary drug resistance in previously untreated individuals.

Drug resistance is strongly associated with previous treatment. Previous anti-TB treatment increased the chance of multidrug resistance five-fold (OR 5.41), and MDR-TB occurred significantly more frequently in people aged 25–44 years (OR 2.5) and 45–64 years (OR 1.89). Alcohol abuse (OR 1.56) is also an independent risk factor for MDR-TB due to its impact on TB treatment adherence. Among patients younger than 25 years, female sex (OR 7.81) and place of birth outside the host country (in data from Estonia), mainly in individuals from the former Soviet Union (OR 79.7), were strongly associated with MDR-TB [34]. Immigration, mainly from countries with a high MDR-TB prevalence, could increase the risk of being infected by a resistant strain not only among foreign-born individuals but also among native individuals.

The independent variables associated with XDR-TB were: previous anti-TB treatment (OR 4.01) and homelessness (OR 3.35). Homeless people, who live in poor conditions and are malnourished,

usually have reduced access to healthcare assistance, prolonging the period of infectiousness and, consequently, increasing the risk of mycobacterial transmission among their close contacts.

Other important issues, frequently described by several investigators, are the high rate of defaulting from treatment, and treatment failure among socially disadvantaged patients, including alcohol abusers and homeless people.

Previous reports have described the association between MDR-TB and HIV infection, and a European meta-analysis described a higher risk (OR 3.52) of developing active MDR-TB disease in HIV patients [35, 36]. High TB prevalence in prisons, transmission of resistant strains related to overcrowding and an inability to isolate resistant cases are well documented internationally (the relative risk for MDR-TB is 1.9 [13]).

Epidemiology of MDR-TB

In 2010, there were an estimated 650,000 cases of MDR-TB. Estimates of the proportion of new and retreatment cases that have MDR-TB are summarised in figures 5 and 6 [37].

Up to 2010, 12 countries had reported nationwide or subnational proportions of MDR-TB of 6% or more among new TB cases. Five of these countries also report MDR-TB proportions of 50% or more among previously treated cases. All of these settings are located in the eastern part of Europe or in central Asia. China has reported the results of its first ever nationwide drug resistance survey, with documented proportions of MDR-TB of 5.7% among new cases and 25.6% among those previously treated. This survey confirms previous estimates that about 100,000 MDR-TB cases are emerging in China annually [3, 4].

A recent WHO study showed that over 50% of TB cases in Minsk (Belarus) are affected by MDR-TB [37–39].

A recurring and important question is whether the number of MDR-TB cases is increasing, decreasing or stable [3]. Based on data available from 37 countries, the proportion of MDR-TB among new TB cases appears to be in decline after peaking in the two Russian oblasts of Tomsk (in 2004) and Orel (in 2006), probably reflecting the success of TB control efforts. Similar declines have been documented in Hong Kong (China), Estonia, Latvia, Lithuania and the USA.

The Global Plan includes the target that by 2015 all new cases of TB considered at high risk of MDR-TB should be tested for drug susceptibility (estimated at about 20% of all new cases), including 100% of retreatment cases [40]. With the notable exception of the European Region,

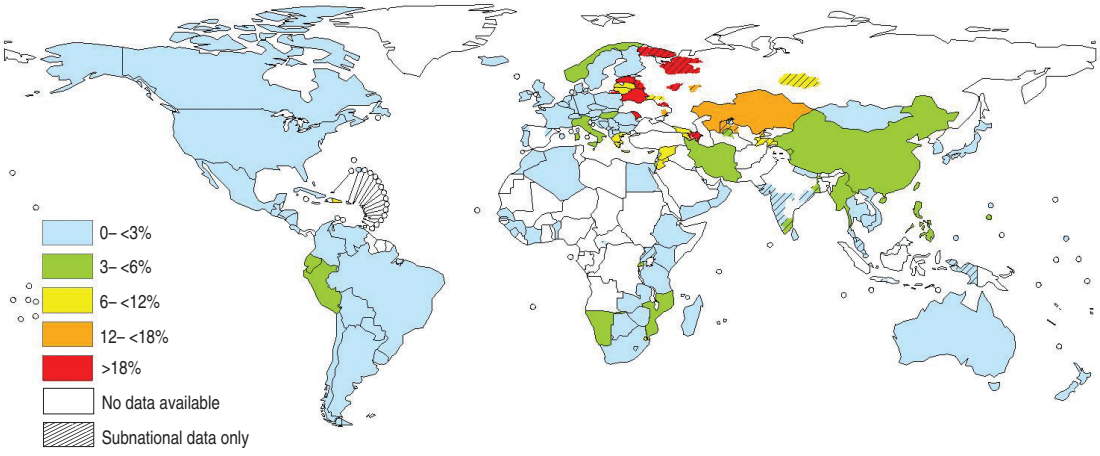

Figure 5. Proportion of multidrug resistance among new tuberculosis cases. Latest available data, 1994–2010. Reproduced and modified from [37] with permission from the publisher.

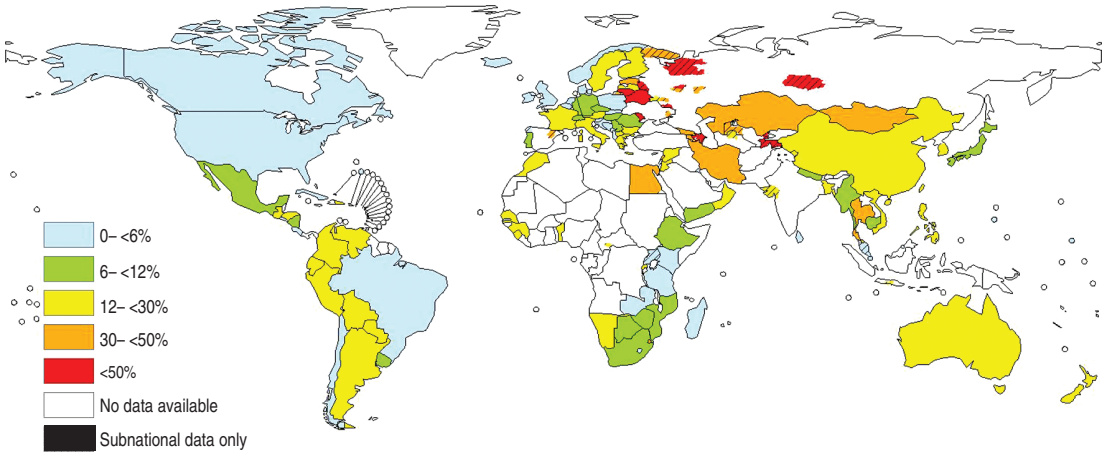

Figure 6. Proportion of multidrug resistance among previously treated tuberculosis cases. Latest available data, 1994–2010. Reproduced and modified from [37] with permission from the publisher.

drug-susceptibility testing (DST) for first-line drugs was performed for only a small proportion of notified cases in 2010. Globally, less than 2% of new cases and 6% of retreatment cases were tested for MDR-TB, with particularly low levels of testing in the South-East Asia and Western Pacific regions. In the European region, 51% of retreatment cases and 30% of the new cases notified in 2010 were tested for MDR-TB. Among the 27 countries with a high MDR-TB burden, the proportion of notified cases that were tested was relatively high in 13 of the 15 European countries that reported data, ranging from 3% of new cases in Tajikistan to 79% of new cases in Estonia, and from 23% of retreatment cases in Tajikistan to over 90% of retreatment cases in Belarus, Latvia and Ukraine [3].

Of the estimated 290,000 cases of MDR-TB among notified cases of pulmonary TB in 2010, only around 50,000 were reported to have been enrolled to receive treatment. China and India account for 44% of the estimated cases (about 130,000) but reported only small numbers of cases as enrolled to receive treatment (just over 4000). In Russia, which ranks third in terms of the estimated number of cases of MDR-TB among notified cases of pulmonary TB (about 31,000 cases), almost 14,000 patients were enrolled to receive treatment. In European countries excluding Russia, there were an estimated 22,000 people with MDR-TB among notified cases of pulmonary TB (8% of the global total) in 2010, just under 19,000 of whom were enrolled to receive treatment. Kazakhstan enrolled more cases to receive treatment (5,705, 13% of the total) than any other country apart from Russia. With 5,402 patients enrolled to receive treatment in 2010, South Africa ranked third. The funding available for MDR-TB treatment in 106 countries that reported data increased from US$0.2 billion in 2006 to US$0.7 billion in 2011. Second-line drugs accounted for 30–50% of the total, depending on the year.

5.4% of MDR-TB cases were found to have XDR-TB. Out of the eight countries reporting XDR-TB in more than 10% of MDR-TB cases, six were located in Eastern Europe and Central Asia [3].

National data on treatment outcomes for cases of MDR-TB are limited. Rates of treatment success are variable, ranging from below 50% (in Moldova, South Africa and Romania) to 74% (in Kazakhstan). Most of these countries thus remain far from the Global Plan target of a 75% treatment success rate as a result of high frequencies of treatment failure, death and default [4, 40].

TB/HIV co-infection

TB and the HIV pandemic are closely interlinked [16–18, 41]. Untreated HIV infection leads to progressive immunodeficiency (measured by a decrease in CD4+ T-lymphocyte count) and

increased susceptibility to infectious diseases, including TB. The pathogenesis of the interaction is an HIV-mediated increase of the risk of progression of *M. tuberculosis* infection to TB disease. Compared with an individual who is not infected with HIV, a person infected with HIV has a 21–34-fold increased risk of developing TB. This risk increases with increasing immunosuppression; in fact, TB can occur at any point in the course of HIV infection, but is much more likely with low CD4+ cell counts. However, in individuals infected with HIV, the development of TB allows HIV to multiply more quickly, resulting in more rapid progression of HIV disease [6, 12, 16–18, 41].

The consequence of the interaction is dual. On the one hand, TB is a leading cause of morbidity and mortality in populations with high HIV prevalence: in areas where the prevalence of TB is also high, one-third or more of HIV infected people may develop TB and eventually die because of it. On the other hand, HIV is driving the TB epidemic in many countries, especially in sub-Saharan Africa and, increasingly, in Asia and South America. For example, some parts of sub-Saharan Africa have seen a three- to five-fold increase in the number of TB case notifications following the rise in HIV prevalence, and HIV seroprevalence in these TB patients is typically up to 75% [4].

Therefore, TB programmes and HIV/AIDS programmes share mutual concerns. Prevention of HIV should be a priority for TB control; TB care and prevention should be a priority of HIV/AIDS programmes [41]. Previously, TB programmes and HIV/AIDS programmes have largely pursued separate courses. However, a new approach to TB control in populations with high HIV prevalence requires collaboration between these programmes. A strong impact on general health services is also demonstrated: the increased case load due to HIV and TB interaction further stretches an overloaded health system causing shortages of staff, medicines and financial resources in general.

The impact of HIV exposes new weaknesses in TB control programmes. The rise in TB suspects is putting a strain on diagnostic services. Extrapulmonary and sputum smear-negative pulmonary TB cases, which are more difficult to diagnose, account for an increased proportion of total cases. There are more adverse drug reactions. There is a higher morbidity and mortality, partly due to other, curable, HIV-related infections. The risk of TB recurrence is higher [16–18, 41].

Epidemiology of TB/HIV co-infection

Beginning in the 1980s, the HIV epidemic led to a major upsurge in TB cases and TB mortality in many countries that persisted throughout the 1990s and up to around 2004, especially in southern and east Africa. Globally, just over one in 10 of the almost 9 million people who develop TB each

Table 1. World Health Organization-recommended collaborative tuberculosis (TB)/HIV activities

Establish and strengthen the mechanisms for delivering integrated TB and HIV services
Set up and strengthen a coordinating body for collaborative TB/HIV activities functional at all levels
Determine HIV prevalence among TB patients and TB prevalence among people living with HIV
Carry out joint TB/HIV planning to integrate the delivery of TB and HIV services
Monitor and evaluate collaborative TB/HIV activities
Reduce the burden of TB in people living with HIV and initiate early ART (the Three I's for HIV/TB)
Intensify TB case-finding and ensure high-quality anti-TB treatment
Initiate TB prevention with isoniazid preventive therapy and early ART
Ensure control of TB infection in healthcare facilities and congregate settings
Reduce the burden of HIV in patients with presumptive and diagnosed TB
Provide HIV testing and counselling to patients with presumptive and diagnosed TB
Provide HIV prevention interventions for patients with presumptive and diagnosed TB
Provide co-trimoxazole preventive therapy for TB patients living with HIV
Ensure HIV prevention interventions, treatment and care for TB patients living with HIV
Provide ART for TB patients living with HIV

ART: antiretroviral therapy. Reproduced and modified from [41] with permission from the publisher.

year are HIV positive, which is equivalent to 1.1 million new TB cases among people living with HIV in 2010. In the African Region, which accounted for 82% of the new TB cases that were living with HIV in 2010, an estimated 900,000 (39%) of the 2.3 million people who developed TB in 2010 were HIV positive. Globally, in 2010, there were an estimated 0.35 million deaths (range 0.32 million–0.39 million) from TB among people who were HIV positive [3]. WHO, UNAIDS (the Joint United Nations Programme on HIV/AIDS) and the Stop TB Partnership have set a target that by 2015, TB mortality rates among people who are HIV positive should be reduced by 50%, compared with 2004 (the year in which TB mortality among HIV-positive people is estimated to have peaked). WHO has provided clear recommendations about the interventions needed to prevent, diagnose and treat TB in people living with HIV since 2012. The recommended interventions are collectively known as collaborative TB/HIV activities; a list of 12 recommended interventions addressing areas of mutual interest (*e.g.* staff training, public education, drug supply, case detection and management, and surveillance) were released in 2004 and updated in 2012 (table 1) [41].

Conclusions

In spite of the significant achievements of the international community under the WHO guidance and the downward trend the pandemic has initiated, TB is still causing a considerable burden of disability and death globally. Further efforts are necessary to improve the surveillance tools available to describe the global epidemiology of TB, in order to provide quality monitoring and evaluation of control and elimination efforts in line with the Stop TB Strategy to reach the Millennium Development Goals and the Stop TB Partnership targets.

Statement of Interest

None declared.

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Chapter 3

Pulmonary diseases caused by non-tuberculous mycobacteria

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SUMMARY: Pulmonary disease due to non-tuberculous mycobacteria (NTM) is an emerging infection, mainly in regions with a decreasing prevalence of tuberculosis (TB). Patients with existing pulmonary diseases (*e.g.* cystic fibrosis, chronic obstructive pulmonary disease (COPD) and/or bronchiectasis), or patients with local or systemic immunosuppression are at risk of developing NTM lung disease. Disease manifestations can be: fibrocavitary, resembling TB; nodular/bronchiectatic, usually in elderly lean, nonsmoking female patients; or hypersensitivity-like after exposure to contaminated water. Since the clinical relevance of pulmonary NTM isolates differs significantly between NTM species, correct laboratory identification of NTM isolates is important to guide treatment decisions and drug-susceptibility testing (DST) efforts. Diagnosis requires the application of clinical and microbiological criteria according to published American Thoracic Society (ATS)/Infectious Diseases Society of America (IDSA) guidelines. Treatment decisions need to be individualised; long-term antibiotic therapy may be combined with surgical resection of affected portions of the lung.

KEYWORDS: Lung disease, *Mycobacterium abscessus*, *Mycobacterium avium*, *Mycobacterium intracellulare*, *Mycobacterium xenopi*, non-tuberculous mycobacteria

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In settings in which the incidence and prevalence of tuberculosis (TB) have fallen during recent decades, clinicians now face what appears to be an emergence of disease caused by non-tuberculous mycobacteria (NTM), *i.e.* all mycobacteria other than the *Mycobacterium tuberculosis* complex and *Mycobacterium leprae*. The NTM can cause a wide range of infections, of which pulmonary infections are most frequent [1]. Owing to their similarities to conventional pulmonary TB in terms of clinical presentation, and the overlap in diagnostic tools and treatment modalities, pulmonary NTM diseases are mostly diagnosed by physicians who also treat TB patients. Hence, this *European Respiratory Monograph* on TB would not be complete if it did not cover pulmonary NTM disease. In this chapter, we review the entire breadth of this emerging field of medicine, from epidemiology to clinical presentation, treatment and laboratory aspects.

Epidemiology of pulmonary NTM infections

NTM are a group of over 140 different species that can cause a wide array of infections in humans and animals [2]. NTM lung disease is most frequent and represents 65–90% of all clinical NTM disease [1, 3, 4]. There is growing evidence that the incidence of NTM lung disease and associated hospitalisations is on the rise, mainly in regions with a low prevalence of TB [5–10]. In the USA, prevalences of 1.4–6.6 in 100,000 have been measured [5–10]. In parallel, skin sensitisation to *Mycobacterium intracellulare* has also increased in the USA [11]. Factors that may underlie this changing epidemiology are increases in the prevalence of the susceptible host; for example, the number of patients with systemic (*e.g.* HIV infection, haematological malignancy, inheritable disorders of immunity, immunosuppressive drug use including tumour necrosis factor (TNF)-α inhibitor therapy [12], or systemic or inhaled corticoid therapy [13]) or local immunosuppression (*e.g.* pre-existing pulmonary disease, such as cystic fibrosis patients and patients with chronic obstructive pulmonary disease (COPD)) has increased [2, 14]. The growing awareness of the entity of pulmonary NTM disease may contribute to this epidemiological trend. The prevalence of NTM in respiratory specimens differs significantly in different parts of the world (W. Hoefsloot, Radboud University Nijmegen Medical Center, Nijmegen, the Netherlands; personal communication) [15], and changes over time in the isolation frequency of the different NTM species from respiratory specimens or in patients with cervical lymphadenitis have been described [2, 14, 16]. These differences are partly explained by the ever more precise taxonomy of the genus *Mycobacterium*, but they may also be related to changes in environmental exposure [17, 18] or a decrease of cross-protection due to reduced TB prevalence or diminished use of bacille Calmette–Guérin (BCG) vaccination [19–21].

Key laboratory features of NTM

Identification and taxonomy

Correct identification of clinical NTM isolates is important because NTM species differ in their clinical relevance, *i.e.* the percentage of patients from whom the species is isolated who are ultimately considered to have true disease caused by this NTM (fig. 1) [1]. Identification results can thus help determine the level of suspicion of true NTM disease. Treatment regimens and methods of drug-susceptibility testing (DST) also differ according to NTM species, mainly between slowly and rapidly growing species [26].

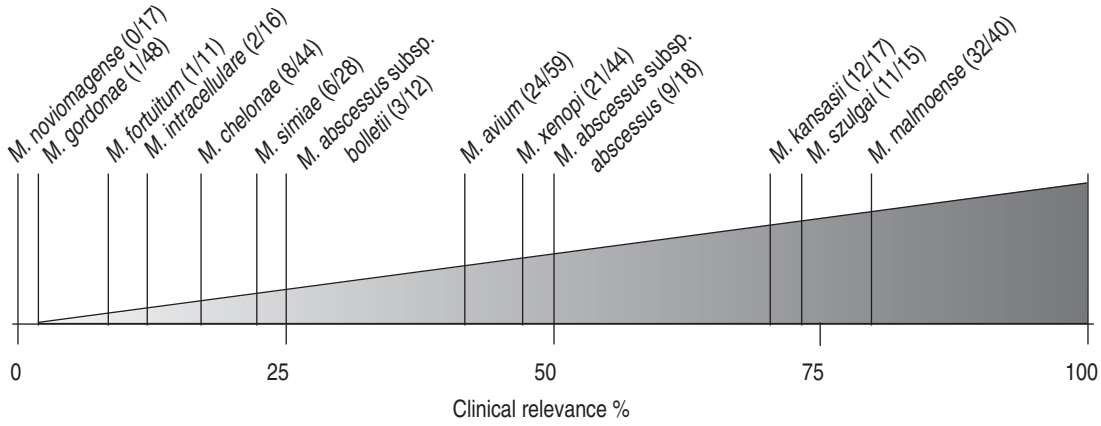

Figure 1. Clinical relevance of common non-tuberculous mycobacteria (NTM) in pulmonary isolates as measured in the Netherlands. Clinical relevance is expressed as the percentage of patients with isolates of the respective species that ultimately met American Thoracic Society (ATS) diagnostic criteria. Numbers in parenthesis indicate the number of true cases/number of patients with the respective NTM isolate. Please note that clinical relevance of certain species may vary in different geographical regions. Data from [1, 22–25].

Laboratory identification of NTM has moved from phenotypic and biochemical analyses to molecular tools, with a huge increase in discriminatory power as a result; all these techniques have their characteristic advantages and disadvantages (table 1). Owing to these molecular tools, including 16S ribosomal DNA (rDNA) gene sequencing, >140 different NTM species have now been described, yet some 20 species make up 95% of all clinical isolates. This "top 20" shows important regional differences (W. Hoefsloot, personal communication) [27, 28]. To identify NTM without the use of sequencers, molecular probes have been designed that can identify multiple species within a single assay (table 1). The latest addition to the identification tools is matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry. This technique is currently being optimised for application in mycobacteriology ([29] and unpublished data).

With the many new species now described, the debate on exact species definitions in the genus *Mycobacterium* continues and the first moves to reassign species as subspecies (*e.g. Mycobacterium bolletii* and *Mycobacterium massiliense* to *Mycobacterium abscessus* subsp. *bolletii*) are now being seen [30].

Drug-susceptibility testing

The role of DST in the choice of agents for the antimicrobial treatment of NTM disease, mainly that caused by slow growers, remains a subject of debate [31]. There are important discrepancies between minimum inhibitory concentrations (MICs) measured *in vitro* and the activity of the drug observed *in vivo* [2, 32–37]. Test methods and conditions have a profound impact on results, and use of the methodology recommended by the Clinical Laboratory Standards Institute [38], despite its inherent limitations, is recommended [2, 27].

For the *Mycobacterium avium* complex (MAC), only susceptibility testing of macrolides (*i.e.* clarithromycin) is currently recommended, because its results have been clinically validated [38, 39]. For *Mycobacterium kansasii*, initial testing should include only rifampicin; rifampicin-resistant isolates have been observed in patients who failed treatment with rifampicin-based regimens [40, 41]. For the rapid growers, relations between MICs and outcomes have been studied for several drugs (*e.g.* tobramycin, co-trimoxazole, cefoxitin and doxycycline), albeit mostly in extrapulmonary disease and key drugs, including amikacin and macrolides, were not included [42]. MICs of any drug other than those mentioned should be interpreted with caution; seeking expert consultation before applying nonstandard drugs in regimens is recommended.

Inducible macrolide resistance owing to ribosomal RNA (rRNA) methylase (*erm*) genes has been demonstrated in many rapid growers, especially in *M. abscessus* subsp. *abscessus*; this inducible resistance is often not reflected in the initial susceptibility results and demands specific testing by laboratories. The relationship between inducible macrolide resistance in *M. abscessus* and the outcome of treatment with macrolide-based regimens remains uncertain [43, 44], although

Table 1. Molecular tools for non-tuberculous myobacterium identification

Type	Commonly used assays/ targets	Discriminatory power	Disadvantages
Single-species DNA probes	AccuProbe (GenProbe, San Diego, CA, USA)	Low, species-specific (4 species)	Low discriminatory power, cost
Line probe assays	GenoType® *Mycobacterium* CM/AS (Hain Lifescience, Nehren, Germany)	Medium (30 species)	Cost
	Inno® LiPA Mycobacteria v2 (Innogenetics, Ghent, Belgium)	Medium (16 species)	Cost, low discriminatory power
PRA	*hsp65*, *rpoB*	Medium–high	Manually processed, error prone
Gene sequence analysis	16S, 16S–23S ITS, *hsp65*, *rpoB*, *secA1*	Very high (all species)	Requires access to sequencers, slow

PRA: PCR product restriction analysis; ITS: internal transcribed spacer.

outcomes seem better in *M. abscessus* subsp. *bolletii* (formerly *M. massiliense*) in which the *erm* gene is not functional [27].

Clinical presentations of pulmonary NTM disease

Four distinct manifestations of pulmonary NTM disease are known: 1) fibrocavitary disease; 2) nodular/bronchiectatic disease; 3) hypersensitivity disease; and 4) the rare solitary pulmonary lesion type that mimics malignancy. Note that these are not absolute and mixed types can occur.

Fibrocavitary disease

In his seminal review of NTM diseases from 1979, WOLINSKY [45] noted that, ''chronic pulmonary disease resembling tuberculosis represents the most important clinical problem associated with NTM'' and that the chest radiograph typically showed ''fibrosis and a thin-walled cavity in the right upper lobe''. The typical patient was middle-aged, male, smoked cigarettes, had underlying chronic lung disease, including chronic obstructive lung disease, pneumoconiosis and/or previous TB, and presented with chronic cough, sputum production and weight loss. As a consequence of the cavitary abnormalities frequently encountered radiographically, the sputum from these patients is usually acid-fast bacilli (AFB) smear and culture positive. Once the diagnosis of TB has been excluded, the diagnosis of fibrocavitary MAC lung disease is relatively straightforward. While the recognised spectrum of NTM lung disease presentation has broadened with the recognition of NTM disease associated with bronchiectasis and nodular densities, the presentation of a typical fibrocavitary MAC lung disease patient has remained remarkably constant. In the USA, slowly growing NTM species such as MAC and *M. kansasii* are the NTM species most often associated with fibrocavitary NTM lung disease; however, other species such as *Mycobacterium szulgai*, *Mycobacterium xenopi* and *Mycobacterium malmoense* are also frequently encountered in other geographic areas, especially northern Europe [2, 22, 23, 35, 46, 47]. As opposed to the USA, this form of NTM lung disease appears to be predominant in northern Europe. Although diagnosis of fibrocavitary NTM disease may not present an especially difficult challenge, the management of these patients can be extremely difficult due to underlying lung disease and limited respiratory reserve with the potential for progressive cavitary lung destruction and respiratory compromise. Although not rigorously described, the available evidence supports the view that this type of NTM disease is associated with relatively high mortality and that these patients require aggressive therapy [22, 23, 35, 47].

Nodular/bronchiectatic disease

In 1989, PRINCE *et al.* [48] convincingly described patients with a progressive noncavitary, nodular/bronchiectatic form of MAC lung disease. In 1992, REICH and JOHNSON [49] proposed the name ''Lady Windermere syndrome'' for this disease manifestation, after the main character in Oscar Wilde's play [50], based on the hypothesis that voluntary cough suppression had a role in the aetiology of the disease. It is now clear that this nodular/bronchiectatic form of NTM lung disease can be seen with essentially any NTM respiratory pathogen, albeit most commonly with MAC, and that in the USA, nodular/bronchiectatic NTM disease is the most commonly encountered form of MAC lung disease [51–53]. Patients with the greatest apparent predisposition for nodular/bronchiectatic NTM lung disease include post-menopausal females who share a distinct morphotype and frequently also carry cystic fibrosis transmembrane conductance regulator (CFTR) mutations [54, 55]. The diagnosis of nodular/bronchiectatic NTM lung disease is guided most importantly by clinical suspicion and then by adherence to published diagnostic guidelines (table 2) [2]). In this setting, shared symptoms of bronchiectasis and nodular/bronchiectatic NTM lung disease, including cough, sputum production, fatigue and weight loss, can impede a timely diagnosis. Similarly, radiographic abnormalities of bronchiectasis may mask or confuse radiographic changes associated with NTM disease, although patterns such as ''tree-in-bud'' abnormalities, nodules and cavitation may raise suspicion of nodular/bronchiectatic NTM disease [56, 57]. Ultimately, microbiology is the

Table 2. Summary of the American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) diagnostic criteria for pulmonary non-tuberculous mycobacteria (NTM) infection

Clinical (all three need to be fulfilled)

1) Pulmonary symptoms;
2) Nodular or cavitary opacities on chest radiograph, or a HRCT scan that shows multifocal bronchiectasis with multiple small nodules; and
3) Appropriate exclusion of other diagnoses.

Microbiological (only one is needed)

1) Positive culture results from at least two separate expectorated sputum samples[#];
2) Positive culture results from at least one bronchial wash or lavage; or
3) Transbronchial or other lung biopsy with mycobacterial histopathological features[¶], and positive culture for NTM or biopsy showing mycobacterial histopathological feature[¶], and one or more sputum or bronchial washing that is culture positive for NTM.

At least three consecutive respiratory samples are needed to apply these criteria. Expert consultation should be obtained when NTM are recovered that are either infrequently encountered or that usually represent environmental contamination. Patients who are suspected of having NTM pulmonary disease but who do not meet the diagnostic criteria should be followed until the diagnosis is firmly established or excluded. Making the diagnosis of NTM pulmonary disease does not, *per se*, necessitate the institution of therapy, which is a decision based on the potential risks and benefits of therapy for individual patients. HRCT: high-resolution computed tomography. [#]: if the results from the initial sputum samples are nondiagnostic, consider repeat sputum acid-fast bacilli (AFB) smears; [¶]: granulomatous inflammation or AFB. Reproduced and modified from [2] with permission from the publisher.

most important element of diagnosis. Clinicians must have familiarity with the pathogenic potential, as opposed to the likelihood of recovery through environment contamination, of NTM species. Diagnostic criteria for respiratory NTM isolates aid in the determination of which NTM isolates are clinically significant. Nodular/bronchiectatic NTM prognosis appears to be one of relatively slow disease progression. While the negative impact of NTM infection on quality of life in this setting is readily apparent, a negative effect on life expectancy has not been established. As has often been said, the diagnosis of nodular/bronchiectatic NTM lung disease should trigger careful evaluation of the microbiological and radiographic data over time in conjunction with the patient's symptoms to make a reasonable decision about therapy based on an individual's risk/benefit assessment.

Hypersensitivity-like disease

Inhalation of mycobacterial antigen through aerosolised contaminated water in hot tubs (usually *M. avium*) as well as metalworking fluid (usually *Mycobacterium immunogenum*) can lead to a hypersensitivity-like disease [58–62]. The ability of mycobacteria to grow across a wide range of temperatures and resistance to disinfectants enables replication [2, 63]. Patients are usually nonsmokers [64], and present with subacute onset of dyspnoea and cough. Fever and hypoxaemia can also occur [58, 61]. Key elements for the diagnosis are compatible clinical history and microbiology. Mycobacteria should be isolated from both patient specimens and hot tub samples (or other potential sources) to confirm the diagnosis [2, 59]. The lung histopathology demonstrates non-necrotising granulomas. Other findings may include necrotising granulomas, organising pneumonia or interstitial pneumonia [58]. Culture of tissue is generally positive for mycobacteria. Computed tomography scans demonstrate infiltrates, centrilobular nodules and ground-glass opacities [61, 65, 66]. The differential diagnosis of hypersensitivity-like mycobacterial disease is often hypersensitivity pneumonitis or sarcoidosis [64]. The cornerstone of treatment is removal of the patient from the antigen. In advanced cases, corticosteroids and/or antimycobacterial therapy may be given [2, 61]. If antimycobacterial therapy is started, it may be given for a shortened period of time (*i.e.* 3–6 months) [2]. Halogen disinfection over ultraviolet light and hydrogen peroxide for hot tubs has been preferred by some [63].

Cystic fibrosis

The best described and specific bronchiectasis-associated disease that is a predisposition for NTM infection is cystic fibrosis. In a large multicentre study evaluating the prevalence of NTM respiratory isolates in cystic fibrosis patients in the USA, it was found that 13% of the cystic fibrosis patients had NTM respiratory isolates, including 72% MAC and 16% *M. abscessus* [67, 68]. The NTM species distribution is reversed in cystic fibrosis patients in Europe, where *M. abscessus* predominates [69]. Published guidelines suggest that NTM isolates may be clinically significant in this setting if other respiratory pathogens are excluded as a possible cause of the patient's clinical deterioration and established diagnostic (microbiological) criteria for NTM disease are met [2]. The applicability of diagnostic guidelines created for non-cystic fibrosis patients is not entirely clear and, to date, no reliable algorithm has emerged that predicts which cystic fibrosis patients with NTM respiratory isolates will have progressive NTM disease and which patients, especially those with MAC respiratory isolates, require therapy directed against the NTM pathogen. The pathogen of most concern is *M. abscessus* due to case reports describing rapid clinical deterioration and even death in some cystic fibrosis patients infected by *M. abscessus* [70]. This concern is, unfortunately, confounded by the difficulty in effectively treating *M. abscessus*, eliminating empirical therapy as a diagnostic tool and resulting in a complicated risk/benefit decision even with established *M. abscessus* disease in the absence of a mechanism for accurately predicting which patients will have disease progression without therapy and those likely to respond favourably to therapy. The clinician is frequently left with the difficult choice between a period of careful clinical observation with the potential for rapid clinical deterioration *versus* initiation of potentially toxic therapy with uncertain clinical benefit. Another potential complication is the recommendation for macrolides as immune modulating agents in cystic fibrosis [71]. Macrolide monotherapy may not only predispose cystic fibrosis patients to mycobacterial infection but can result in the emergence of macrolide-resistant MAC isolates, which severely negatively impacts treatment success of MAC infection [72].

Infections in the immunocompromised host

Manifestations of NTM pulmonary disease in immunosuppressed patients depend on the type and severity of immunosuppression. Patients with systemic immunosuppression (*e.g.* HIV infection, haematological malignancy, immunosuppressive drug use including TNF-α inhibitor therapy [12] or systemic corticoid therapy) are at risk of developing disseminated and localised NTM diseases, whereas patients with local immunosuppression (*e.g.* pre-existent pulmonary disease or inhalative corticoid therapy [13]) are at risk of developing pulmonary NTM disease. The most important examples are summarised as follows.

HIV patients with severe CD4 cell depletion usually present with disseminated NTM disease, of which MAC is the most common. Blood cultures are usually positive. Isolation of the pathogen from respiratory secretions is common even without pulmonary involvement [2]. NTM pulmonary disease as a single NTM manifestation is rare in HIV patients and has been found to be present in 2.5% of 200 patients with disseminated MAC infection [73–76].

30% of HIV patients with NTM-associated immune reconstitution inflammatory syndrome (IRIS) present with thoracic disease [77]. Weeks to months after starting active antiretroviral therapy (ART), patients may develop cough (93%), fever (80%), night sweats (73%) or dyspnoea (47%) [77]. Chest computed tomography often demonstrates lymphadenopathy, tree-in-bud infiltrates, cavitary lesions, nodules or pericardial effusion [77]. Treatment includes continuation of ART and mycobacterial therapy. Recommendations regarding the length of NTM-specific treatment in HIV-associated IRIS are not evidence based. Depending on the CD4 count, some experts would discontinue NTM treatment 6 months after culture conversion [77, 78].

NTM pulmonary disease in haematopoietic stem cell and solid organ transplant (SOT) recipients is rare, with an incidence of 0.2–5% [79–81]. Stem cell transplant recipients often present with

catheter-related infections due to rapid growing mycobacteria, with NTM pulmonary disease being the second most common complication [79]. Graft *versus* host disease appears to be a risk factor for NTM, with the majority occurring within the first half-year post-transplantation. Whereas skin NTM disease has most often been reported in kidney or heart transplant patients, pleuropulmonary disease is most frequently found in lung transplant recipients (>50% of cases) and heart transplant recipients (>25% of cases) [79]. Median time to presentation with NTM infection was later in patients with SOTs (lung, 15 months; kidney, 24 months; heart, 30 months) [79]. Treatment should be instituted according to published guidelines [2, 80]. Interactions with immunosuppressive agents need to be considered [80].

Clinical relevance and diagnostic criteria

Since the NTM are environmental organisms and are present in tap water, humans are probably exposed to NTM on a daily basis. The human airways are thus occasionally contaminated with NTM and this aspect implies that a single positive culture from a sample of a nonsterile body, such as the human airways, is insufficient to diagnose NTM disease.

The American Thoracic Society (ATS) and Infectious Diseases Society of America (IDSA) have issued statements including a set of criteria to differentiate chance NTM isolation from true pulmonary NTM disease, which are summarised in table 2 [2]. To diagnose pulmonary NTM disease, clinical, radiological and microbiological evidence of disease should be gathered. Symptoms are generally nonspecific, in part owing to frequent underlying conditions. Radiological abnormalities are more specific but the most compelling criterion to diagnose NTM lung disease is the microbiological criterion, which was based on the finding that pulmonary disease (infiltrates or cavitary lesions) progressed in 98% of the patients who had two or more positive sputum cultures for MAC, *versus* just 2% in those with a single positive culture during 12 months of observation. For 97% of patients, the first two positive cultures grew from the initial three sputum specimens [82]. These latter findings are less applicable to the nodular/bronchiectatic type of NTM lung disease, because these patients can have less or no sputum production. Bronchoalveolar lavage (BAL) is likely to be more sensitive than sputum culture to diagnose nodular/bronchiectatic NTM lung disease [83]. In a small study of 26 patients with suspected MAC nodular/bronchiectatic lung disease, BAL yielded positive cultures in 13, *versus* only six by sputum cultures [84]. In nodular/bronchiectatic NTM lung disease in patients who do not produce sputum, a single positive culture from BAL, preferably with histological evidence of mycobacterial disease, may be used to diagnose NTM lung disease. This is incorporated in the most recent statement by the ATS and IDSA (table 2) [2]. It needs to be emphasised that a diagnosis of NTM lung disease from a single positive BAL culture is only appropriate in patients who cannot produce additional respiratory samples. Isolation of rare species or species generally considered nonpathogenic (*e.g. Mycobacterium gordonae*, *Mycobacterium terrae* and *Mycobacterium phlei*) in this setting may warrant a conservative approach and repeat bronchoscopy where possible.

Of all NTM cultured from pulmonary samples, *M. kansasii*, *M. szulgai*, *M. malmoense* (in north-western Europe), and the very rare *Mycobacterium shimoidei* and *Mycobacterium heckeshornense* have been most strongly associated with true NTM disease (fig. 1). Solitary isolates of these species from pulmonary samples in patients with no additional evidence of pulmonary NTM disease are very rare [1, 22, 23, 85]. However, isolation of *M. gordonae*, or to a lesser extent *Mycobacterium chelonae* and *Mycobacterium simiae*, is rarely associated with clinical disease [1, 34, 86]. For these species, solitary isolates from pulmonary samples without additional evidence of NTM disease are the rule rather than exception. MAC and *M. xenopi* seem to form an intermediate category, as 40–70% of all isolates are considered clinically relevant in different studies [1, 87, 88]. To prevent unwarranted diagnoses and treatment of NTM disease as well as unnecessary diagnostic delay, it could be helpful to use separate, more stringent criteria for species of low clinical relevance, and less stringent criteria for species of high clinical relevance.

Treatment of NTM lung disease due to common pulmonary NTM species

In contrast with TB, diagnosis of NTM lung disease does not necessarily require specific treatment. The decision to treat needs to be individualised, depending on the specific NTM species, patient acceptance, tolerance and adherence, and treatment goals (reduction of symptoms or sputum conversion). Treatment modalities may include observation with best pulmonary care, intermittent antibiotic treatment, oral antibiotics three times a week or daily, additional intravenous therapy for several months, or surgical therapy [2].

Antibiotic therapy

There are several obstacles peculiar to NTM that impede effective antibiotic therapy. As discussed earlier, *in vitro* susceptibility testing is frequently not a guide for effective *in vivo* response to antibiotics. One overriding therapeutic imperative is to avoid the emergence of macrolide-resistant MAC [89] or *M. abscessus* [27] strains during therapy. Still, for unknown reasons, the chance of treatment success for MAC lung disease is greatest with the first treatment effort even without the development of macrolide resistance [32–34, 36]. Additionally, in patients who are adequately treated, subsequent isolation of MAC is more likely to represent ''re-infection'' with a new MAC genotype than disease ''relapse'' with isolation of the pre-treatment MAC genotype [90]. The clinical significance of re-infection MAC isolates must be individually determined. For *M. abscessus*, no reliably and predictably effective treatment exists. If antimicrobial therapy is administered, two parenteral agents and a macrolide, if appropriate (*i.e.* if the *M. abscessus* isolate does not have inducible *erm* gene activity), should be used [91].

The goal of therapy is 12 months of sputum culture negativity while on therapy. The recommended treatment regimens for selected NTM respiratory pathogens are listed in table 3 [2]. These multidrug regimens lead to significant pharmacokinetic interactions. In particular, rifampicin lowers the serum levels of macrolides and moxifloxacin in patients with NTM pulmonary disease [92].

Table 3. Recommended treatment regimens for selected non-tuberculous mycobacteria (NTM) respiratory pathogens

NTM	Regimen
MAC	Macrolide (azithromycin or clarithromycin), rifamycin and ethambutol daily or three times a week, with or without an injectable agent three times a week
Mycobacterium kansasii	Rifampicin, ethambutol and isoniazid daily, or rifampicin, a macrolide and ethambutol daily or three times a week
Mycobacterium szulgai	Macrolide (azithromycin or clarithromycin), rifamycin and ethambutol daily or three times a week, with or without an injectable agent three times a week
Mycobacterium malmoense	Macrolide (azithromycin or clarithromycin), rifamycin and ethambutol daily or three times a week, with or without an injectable agent three times a week
Mycobacterium xenopi	Macrolide (azithromycin or clarithromycin), rifamycin and ethambutol daily or three times a week, with or without an injectable agent three times a week
Mycobacterium simiae	No regimen of proven value
Mycobacterium abscessus	
M. abscessus subsp. *abscessus*	Three or four of the following: amikacin, cefoxitin, imipenem, tigecycline, linezolid or a macrolide[#]
M. abscessus subsp. *bolletii*	A macrolide[#] plus two of the following: amikacin, cefoxitin, imipenem, or linezolid

MAC: *Mycobacterium avium* complex. [#]: may be inactive if *erm* gene is functional.

The clinical implications of these low serum levels remain unknown but they may partly explain the poor outcomes of drug treatment.

Treatment outcomes differ according to species; in most settings, the best outcomes are seen in *M. kansasii* and *M. malmoense*, slightly worse outcomes are seen in MAC, and very poor outcomes are recorded in patients with NTM pulmonary disease caused by *M. xenopi*, *M. simiae* and particularly *M. abscessus* subsp. *abscessus* [76, 87].

Surgery

The potential benefits of surgery should be considered for every individual patient in whom NTM pulmonary disease is diagnosed and re-evaluated during treatment. Cavitary disease, destroyed lung tissue and continued sputum positivity despite maximal drug therapy have been proposed as indications for adjunctive surgery in NTM pulmonary disease [93, 94]. Surgery should be given full consideration at the outset of treatment plans of patients with select localised rapidly growing mycobacteria (*e.g. M. abscessus* subsp. *abscessus*) in which medical therapy alone has been particularly daunting. Lobectomy or bilobectomy is a possibility if cavitary lesions in an upper lobe are accompanied only by minor nodular lesions in other areas of lung. In eligible patients who present with a destroyed lung, pneumonectomy is the procedure of choice.

Adjunctive surgical treatment for NTM lung disease yields encouraging results in the few published case series. Conversion rates are generally very high (90–100%) and few relapses are noted, marking the efficacy of combined medical and surgical treatment [93, 94]. These positive results underscore the importance of continuing medical therapy before and after surgical intervention for optimal success. The recent experiences with a video-assisted thoracoscopic approach have been very positive and may yield lower complication rates [95]. For all surgical procedures, careful patient selection based on the extent and type of disease, and on cardiopulmonary fitness, is of critical importance. Moreover, surgery for mycobacterial disease should be performed by experienced thoracic surgeons in centres that can offer long-term follow-up including continuation of drug treatment with an effective regimen.

Conclusion

NTM pulmonary disease has emerged as an increasingly important subject in medicine in countries with a low prevalence of TB. Because its importance has only been perceived in the past two decades and this disease has remained relatively rare, a large number of undiagnosed NTM pulmonary disease cases certainly exist among patients with chronic pulmonary disease, especially COPD. Every effort has to be made to further increase the awareness and knowledge of the diagnosis and therapy of NTM pulmonary disease among respiratory specialists who care primarily for patients with chronic pulmonary diseases. In addition, little clinical research has been performed and, as a result, treatment regimens have a very limited evidence base. The exact pathogenesis of NTM lung disease also remains largely unknown. These issues require urgent attention from pulmonologists, microbiologists, immunologists and basic scientists.

With increased international cooperation, necessary and adequately powered clinical trials can be conducted. New drugs and combinations, as well as optimal dosing of drugs in current regimens to counter pharmacokinetic interactions, should be the subject of trials. Optimal regimens for nodular/bronchiectatic disease may differ from those in cavitary disease and separate trials are probably helpful to address this issue. Although the poor outcomes of current treatment regimens are frustrating, the future challenges of developing new regimens, and unravelling the exact pathogenesis and the intricacies of diagnosing and treating NTM pulmonary disease in individual patients, often with many comorbidities, render this a particularly exciting and evolving field of medicine.

Statement of Interest

None declared.

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Chapter 4

Human genetic variability and susceptibility to pulmonary TB

Thorsten Thye and Christian G. Meyer

SUMMARY: Over the last few decades, the observed impact of genetic variation on infectious disease phenotypes has contributed to the understanding of why individuals exist who, when infected with the same pathogen, may resist infections, while others experience severe disease or even may succumb to the infection. Since the early recognition of the protective effect that the sickle cell trait exerts on courses of *Plasmodium falciparum* malaria, studies of genetic susceptibility to infectious disease in humans have, through rapid technological advancements and the availability of analytical tools, made enormous progress. This also applies to investigations of host genetic factors in tuberculosis (TB), where considerable efforts have been undertaken. The methodologies for the identification of genetic variants comprise a variety of techniques for genotyping. In addition to genome-wide linkage and candidate gene studies, whole-genome and high-throughput DNA sequencing and appropriate appliances for genome-wide association studies, such as high-density single-nucleotide polymorphism arrays, are now available. In this chapter, we provide a brief overview of the genetic epidemiology of pulmonary TB.

KEYWORDS: Candidate gene studies, genome-wide association studies, genome-wide linkage studies, tuberculosis susceptibility

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Evidence of genetic factors that determine the phenotype arising after an infection with *Mycobacterium tuberculosis* and the justification for conducting genetic studies in tuberculosis (TB) comes from early observations of an assumed heritability of susceptibility to TB. More generally, the genetic and socio-environmental impact on mortality due to infectious disease of adult individuals has been studied and compared in biological children and adoptees. With regard to infectious diseases, the risk of dying from the same cause was significantly increased among biological children when compared with adoptees, with an odds ratio of 5.81 (95% CI 2.47–13.7), indicating, in addition to environmental factors, a relevant genetic background of disease phenotypes [1]. Earlier studies in families with one or more individuals affected by TB looked at

the incidences among relatives. However, initial attempts to demonstrate a genetic role of TB in families were not convincing [2].

The individual immune responses to an infection with *M. tuberculosis* may differ considerably. This became evident in a vaccination accident in 1928 in Lübeck, Germany. A virulent *M. tuberculosis* strain was administered as a vaccine to 251 newborns and caused TB in 212 of the children; subsequently, 77 newborns died [3]. Although all children had received equal doses of virulent mycobacteria, 39 of the newborns did not develop any symptoms of TB, which was attributed to a distinct genetic make-up that resulted in protective innate and adaptive immune responsiveness.

Twin studies and comparisons of monozygotic *versus* dizygotic twins with similar or, even better, identical social, environmental and exposure conditions hold a more promising potential for the substantiation of genetic effects on TB susceptibility. Based on early twin studies [4, 5], in the USA, KALLMANN and REISNER [6] described a higher concordance of TB among 78 pairs of monozygotic twins, when compared with 230 pairs of dizygotic twins. The concordance in monozygotic and dizygotic twins was 66% and 23%, respectively, whereby the concordance in dizygotic twins was similar to that among non-twin siblings. Although substantially biased and limited by several weaknesses in the design of the study, it strongly supported the concept of genetic contributions to TB susceptibility. The inherent limitations of the study became evident later, when a comparable design, but taking into account all flaws of the KALLMANN and REISNER [6] study, was applied (referred to as the Prophit Survey) [7]. The higher TB concordance among monozygotic twins was attributed to various factors, in particular: to a higher rate of common residence of monozygotic twins and higher concordance among these pairs; to the higher rate of female twin pairs in the study (although it is known that males are more prone to TB than females); to a higher rate of sputum positivity in index cases indicating an increased potential of infectivity; and to higher rates of TB among parents of monozygotic twins.

The original twin data of the Prophit Survey were again applied in regression models with corrections for age, sex of twin pairs, and distinct properties of TB, such as the type of disease, detectability of mycobacteria in sputum samples and TB contacts of co-twins [8]. A significant difference in the concordance between mono- and dizygotic twins was confirmed.

Ethnic differences in the occurrence of TB, evidenced by a higher susceptibility of individuals of African descent compared to those of European ancestry [9], also points to a substantial contribution of the genetic architecture in TB susceptibility.

Genetic epidemiology

The role of variable host genetic factors in disease can be studied by means of genetic epidemiology and statistical genetics. This involves the determination of environmental factors, including socio-economic conditions, and, desirably, if possible and so designed, analyses of a potential interplay between host and pathogen genetic factors. This approach has successfully been applied in studies of genetic factors and TB susceptibility in a sizable TB case–control study from Ghana and has allowed the conception of evolutionary hypotheses on a distinct haplotype of the gene encoding mannose-binding lectin (*MBL*), as will be shown on the example of infections caused by *Mycobacterium africanum* [10]. The classical definition of genetic epidemiology as "a science which deals with the etiology, distribution, and control of disease in groups of relatives and with inherited causes of disease in populations" by MORTON [11] in 1982 has been reaffirmed in 2006 [12]. This definition, which is still valid today, implies that genetic factors can be analysed in families and populations to assess genetic components of a distinct condition, to determine the size of a genetic effect in a disease and, eventually, to identify causative genes or causal genetic variation of a gene.

In addition to family aggregation studies, which are often based on descriptive epidemiology rather than on molecular genetics, and try to identify common genetic components and the roles of environmental factors, the mode of inheritance of a susceptibility factor may be identified through segregation (linkage) studies. The most powerful tools are the various designs of

association studies, in particular analyses of candidate gene variation and genome-wide association studies (GWASs) in case–control groups.

While strong effects are expected and may be detected in monogenic diseases, for example, as shown in most cases of inherited deafness [13–15] and in other conditions, the dissection of complex multigenic traits may pose considerable problems. When many genes and variants contribute to a disease phenotype and exert only weak effects, the relative contributions of genes and the precise identification of the truly causal gene or its variant may be intricate.

This chapter aims to address the most important results of studies of human genetic factors and susceptibility to TB (table 1).

Genome-wide linkage studies

Genome-wide linkage studies bear the potential of defining in which chromosomal region a putatively responsible genetic factor is located. This is achieved through tests of co-segregation of genetic markers and disease loci in affected pedigrees and optimally indicates major susceptibility genes. This approach has been especially useful in monogenic diseases [76].

In the search for chromosomal regions harbouring TB susceptibility genes, only a few studies have applied genome-wide linkage approaches. In a first two-stage genome-wide linkage analysis involving sib-pair families with 92 sib-pairs and, in a second step, 81 sib-pairs from South Africa and the Gambia, suggestive evidence of linkage of markers on chromosome 15q and Xq was obtained. However, the lod scores of 2.00 and 1.77, respectively, were not convincing [77]. In a study of 22 Brazilian TB families, susceptibility regions on chromosomal regions 10q26.13, 11q12.3 and 20p12.1 were reported, also with low lod scores [78]. In a subsequent study of 96 TB-affected Moroccan families, a region on 8q12-q13 was linked to TB susceptibility (lod score 3.94, $p=10^{-5}$) in a subsample of the 96 families (n=39) in which one of the parents of the affected sibs was also affected [79].

Independent mapping of the chromosomal regions 6p21–6q23 and 20q13.31–20q13.33 in West African populations after their identification in a linkage study pointed to the involvement of two genes, melanocortin 3 receptor (*MC3R*) and cathepsin Z (*CTSZ*), in susceptibility to TB [80].

A microsatellite genome scan with 193 participating Ugandan families was performed. Suggestive evidence for linkage was reported for chromosome 7p22–7p21 and the findings on chromosome 20q13 reported by COOKE *et al.* [80] were replicated (p=0.002). In that study, linkage to several candidate genes (*SLC11A1*, *IL1*, *IL12BR2*, *IL12A* and *IFNGR2*; see section on IL-12A later) was also observed [81]. In a following analysis of 93 affected Thai pedigrees with 195 affected individuals, a 5q chromosomal region appeared to be linked to TB susceptibility (lod score 2.29, p=0.0005) [82]. Attempts to stratify for the age of onset of TB were not convincing. The study reported, as did the linkage study from Brazil [78], on suggestive linkage of TB to a chromosome 20p12 region.

A genome-wide linkage study among 128 South African TB families with 350 siblings found the *TST1* (tuberculin skin test 1) locus on chromosome 11p14 ($p=1.4\times10^{-5}$) to control TST reactivity. A second locus (*TST2*) was identified at 5p15 ($p<1\times10^{-5}$), harbouring the potential candidate gene *SLC6A3* that encodes the dopamine transporter DAT1 [83]. This gene and possible associations with TB, to date, have not been studied further.

Notably, the loci defined by genome-wide linkage analyses are still inconsistent across the studies, which again indicates the obstacles encountered when analysing complex disease traits by linkage studies.

Candidate gene studies

A candidate gene approach in infectious and noninfectious diseases focuses on presumed relationships of distinct disease phenotypes with variable genetic elements of a pre-specified gene,

Table 1. Results of studies of human genetic factors and susceptibility to tuberculosis

Gene	Variant	SD	Population	Cases n	Controls n	p-value	GT	OR	[Ref.]
ALOX5	VNTR		Ghana	1916	2269	0.026	(VNTR)5	1.19	[16]
	rs2228065 (g.760)		Ghana	1916	2269	0.026	G>A	1.21	[16]
CR1	rs56393589 (Q1022H)		Malawi	514	913	0.028	TT *versus* GG	3.12	[17]
CTLA4	rs3087243 (+6230)		Ghana	2010	2346	NS			[18]
CXCL10	rs56061981 (-135)		China	240	176	0.01	AA/GA *versus* GG	0.51	[19]
DC-SIGN	rs735239 (-871)		South Africa	351	360	8.2×10^{-4}	A>G	1.85	[20]
	rs735239 (-871)		Tunisia	138	140	NS			[21]
	rs4804803 (-336)	MA	10 studies			NS			[22]
IFNG	rs2430561 (+874)	MA	19 studies			$<1 \times 10^{-4}$	A>T	1.51	[23]
	rs2069705 (-1616)		West Africa	682	619	0.008	G>A	1.49	[24]
	rs2069718 (+3234)		West Africa	682	619	0.009	T>C	1.4	[24]
IFNGR1	Microsatellite, intron 1		Croatia	120	87	0.041	Allele 192		[25]
			The Gambia	351	315	NS			[26]
			Indonesia	382	437	0.01	CA_{12}/CA_{12}	0.5	[27]
	rs2234711 (-56)		West Africa	682	619	0.041	T>C	0.75	[24]
IFNGR2	rs2834213		Vietnam	832	506	0.00054	A>G	0.7	[28]
IL1B	rs16944 (-511)		The Gambia	335	298	0.015	C	0.58	[29]
	rs1143634 (+3953)		Colombia	122	166	0.001	T	0.3	[30]
IL1B/IL1Ra	86-bp VNTR/3953 haplotype		India	89	114	0.028			[31]
IL8	rs4073 (-251)		Whites	106	107	<0.01	T>A	3.41	[32]
	rs4073 (-251)		African–Americans	180	167	<0.01	T>A	3.46	[32]
	rs4073 (-251)		The Gambia	363	320	NS	T>A		[33]
IL10	rs1800896 (-1082)	MA	18 studies			NS			[34]
		MA	5 studies, Europeans			0.01	GA+AA *versus* GG	0.55	[34]
	rs1800871 (-819)	MA	9 studies			NS			[34]
	rs1800872 (-592)	MA	10 studies			NS			[34]
IL12A	rs2243115		China	522	527	0.021	TG+GG *versus* TT	0.67	[35]
IL12B	Microsatellite, intron 2		China	516	514	0.001	$(ATT)_8$	2.14	[36]
IL12RB1	rs11575934, rs375947, rs401502 haplotype		Japan	98	197	0.013	R214-T365-R378	2.45	[37]
	rs11575934, rs375947, rs401502 haplotype		Morocco	101 families		NS			[38]
	rs393548 (-111)		Morocco	101 families		0.013	A>T	2.69	[38]
	rs436857 (-2)		Morocco	101 families		0.019	C>T	2.03	[38]
IRGM	rs9637876 (-261)		Ghana	2010	2346	4.5×10^{-3}	C>T	0.66	[39]
	rs4958842 (-1208)		China	216	275	0.042	G>A	0.58	[40]
	rs10065172		African–Americans	370	180	0.01	C>T	1.54	[41]
	rs10065172		Caucasians	177	110	NS	C>T		[41]
MARCO	rs17009726		China	923	1033	1.10×10^{-4}	A>G	1.57	[42]
MBL2	rs5030737 (R52C)	MA	12 studies			NS			[43]
	rs1800450 (G54D)	MA				NS			[43]
	rs1800451 (G57E)	MA				NS			[43]
	rs1800451 (G57E)		Ghana	477	2236	0.008	G>A	0.6	[10]
MCP1	rs1024611 (-2581)	MA	Africans			$<1 \times 10^{-5}$	A>G	0.79	[44]
		MA	Asians			0.04	A>G	1.84	[44]
		MA	Latin Americans			0.06	A>G	1.9	[44]
	rs2857656 (-362)		Ghana	1964	2312	1.7×10^{-5}	G>C	0.83	[45]
MHC	HLA class I B13	MA	22 studies			$<1 \times 10^{-4}$	B13	0.64	[46]
	HLA class II DR3	MA	22 studies			0.002	DR3	0.72	[46]
	HLA class II DR7	MA	22 studies			$<1 \times 10^{-4}$	DR7	0.65	[46]
	HLA class II DR8	MA	22 studies			0.003	DR8	1.72	[46]
NOD2	rs2066842 (P268S)		African–Americans	377	187	0.02	C>T	0.55	[47]
	rs139104022 (R702W)		African–Americans	377	187	0.01	C>T	0.27	[47]
	rs5743278 (A725G)		African–Americans	377	187	0.03	G>A	2.16	[47]
	rs1861759 (R587R)		China	219	215	2.3×10^{-3}	TT *versus* GG	2.28	[48]
NOS2A	(CCTTT)n microsatellite		Colombia	114	304	0.005	8–11 *versus* 12–16 repeats	0.63	[49]
	rs2779249 (-1026)		Brazil	92 families		0.039	G>T		[50]

Table 1. Continued.

Gene	Variant	SD	Population	Cases n	Controls n	p-value	GT	OR	[Ref.]
	rs2301369 (-2447)		Brazil	92 families		0.029	C>G		[50]
	rs9282799–rs8078340 haplotype		South Africa	431	482	0.038	C-C		[51]
	rs9282799–rs8078340 haplotype		South Africa	431	482	0.029	C-T		[51]
	rs2274894		African–Americans	279	166	0.003	G>T	1.84	[52]
	rs7215373		African–Americans	279	166	0.004	C>T	1.67	[52]
P2X7	rs3751143 (-1513)	MA	7 studies			$<1 \times 10^{-5}$	A>C	1.44	[53]
	rs2393799 (-762)	MA	5 studies			NS			[53]
PTPN22	rs2476601 (R620W)		Colombia	113	161	0.04	C>T	0.3	[54]
	rs2476601 (R620W)		Morocco	123	155	0.01	C>T	0.14	[55]
	rs33996649 (R263Q)		Morocco	123	155	0.01	G>A	5.85	[55]
SLC11A1	rs34448891 (5′-(GT)n)	MA	12 studies (all)				3 alleles *versus* other	1.31	[56]
		MA	Africans					1.28	[56]
		MA	Asians					1.43	[56]
		MA	Caucasians					1.15	[56]
	rs3731865 (INT4)	MA	20 studies (all)				CC+CG *versus* GG	1.23	[56]
		MA	Africans					1.5	[56]
		MA	Asians					1.3	[56]
		MA	Caucasians					1.07	[56]
	rs17235409 (D543N)	MA	29 studies (all)				AA+AG *versus* GG	1.25	[56]
		MA	Africans					1.37	[56]
		MA	Asians					1.18	[56]
		MA	Caucasians					1.48	[56]
	rs17235416 (3′-UTR-del4)	MA	30 studies (all)				TGTG- *versus* TGTG+	1.35	[56]
		MA	Africans					1.23	[56]
		MA	Asians					1.36	[56]
		MA	Caucasians					1.42	[56]
SP110	rs3948464		West Africa	420 families		0.0002	C>T		[57]
	rs2114592		West Africa	420 families		5×10^{-6}	C>T		[57]
	rs3948464/rs2114592		Ghana	2004	2366	NS			[58]
	rs3948464/rs2114592		South Africa	381	417	NS			[59]
	rs3948464/rs2114592		Russia	1912	2104	NS			[60]
	rs3948464/rs2114592		India	110	78	NS			[61]
	rs3948464/rs2114592		Indonesia	351	364	NS			[62]
TIRAP	rs8177374 (S180L)	MA	9 studies	675	605	NS			[63]
	rs7932766 (C558T)		Vietnam	358	392	<0.001	C>T	2.25	[64]
TLR2	Intron II repeat		Korea	176	196	0.02	⩽16 *versus* >16		[65]
	rs5743708 (R753Q)		Turkey	151	116	0.022	AA *versus* GA/GG	6.04	[66]
	rs6265786 (R677W)		Tunisia	33	33	1×10^{-4}	CC *versus* CT		[67]
	Insertion/deletion -196– -174		Caucasians	237	144	7×10^{-4}	II *versus* ID and DD	0.41	[68]
TLR8	rs3764880 (M1V)		Indonesia	375	387	0.007	G>A	1.8	[69]
	rs3764880 (M1V)		Russia	1837	1,779	0.03	G>A	1.2	[69]
TLR9	rs352143/rs5743836		Guinea-Bissau	321	346	NS			[68]
			African–Americans	295	179	NS			[68]
			Caucasians	237	144	NS			[68]
TNF	rs1800629 (-308)	MA	18 studies			NS			[70]
TNFRSF1B	rs3397		South Africa	429	482	0.049		1.22	[51]
	rs3397		Ghana	640	1158	0.007		1.32	[51]
	rs3397		Ghana/South Africa			3.7×10^{-3}			[51]
	rs1061624 rs5030792 rs3397		Ghana/South Africa			1.1×10^{-4}	Haplotype GTT		[51]
VDR	rs10735810 (*FokI*)	MA	Asian	12 studies		<0.1	ff *versus* FF	2	[71]
		MA	African	5 studies		NS			[71]
		MA	South American	2 studies		NS			[71]
	rs731236 (*TaqI*)	MA	Asian	10 studies		NS			[71]
		MA	African	8 studies		NS			[71]

Table 1. Continued.

Gene	Variant	SD	Population	Cases n	Controls n	p-value	GT	OR	[Ref.]
		MA	South American	2 studies		NS			[71]
	rs7975232 (*ApaI*)	MA	Asian	6 studies		NS			[71]
		MA	African	6 studies		NS			[71]
	rs1544410 (*BsmI*)	MA	Asian	6 studies		<0.1	bb *versus* BB	0.5	[71]
		MA	African	4 studies		NS			[71]
GWASs	rs4331426 (Affymetrix 6.0 array GWAS+replication)		Ghana	2145	5548	3.4×10^{-7}	A>G	1.22	[72]
	Affymetrix 500 k GWAS		The Gambia	1309	1377	2.9×10^{-3}	A>G	1.18	[72]
	Replication		Malawi	178	576	0.23	A>G	1.15	[72]
	rs2057178 (Affymetrix 6.0 array GWAS+replication)		Ghana	2127	5636	2.6×10^{-9}	G>A	0.77	[73]
	Affymetrix 500 k GWAS		The Gambia	1207	1349	4.9×10^{-4}	G>A	0.8	[73]
	Replication		Indonesia	1025	983	0.099	G>A	0.84	[73]
	Replication		Russia	4441	5874	0.02	G>A	0.91	[73]
	Illumina 600 k GWAS		Thailand/Japan	620	1524	NS			[74]
	Affymetrix 100 k GWAS		Indonesia	108	115	NS			[75]

For full details of the genes and variants listed in this table, please refer to the main text. SD: study design; GT: genotype; GWAS: genome-wide association study; VNTR: variable number tandem repeat; HLA: human leukocyte antigen; UTR: untranslated region; MA: meta-analysis; NS: nonsignificant.

the product of which is *a priori* known or hypothesised to have a specific biological/physiological role in a well-defined phenotype [84]. The function of the gene product may be important in, for example, innate or acquired immune responses directed against pathogens, or in other events related to the infection or the individual reaction. Candidate gene studies may also be applied to validate associations observed in previous studies or confirm them in different ethnic groups. The candidate gene approach is, thus, strongly driven by both a sound comprehension of the function of the gene and its product or, at least, a reliable or reasonable expectation of biological pathway events influenced by the gene. In order to achieve judicious results and to assess genuine effects, candidate gene studies require an optimal study design with sufficient statistical power that is only achieved with large study groups, well-controlled phenotypes of disease states, a wide gene coverage, stringent quality control of all steps, and solid statistical operations.

As the knowledge of a gene's function in disease is often incomplete or even rudimentary, the selection of candidate genes and their variants to be genotyped will often be arbitrary. The question to be answered is whether a genetic variant occurs more frequently among individuals with a distinct and well-defined phenotype. This is mainly achieved when comparing disease-affected individuals with healthy controls. Hence, a candidate gene study may be a valuable epidemiological approach to unravel relations between a gene or a genetic variant and a phenotype. Notably, as the true exposure of the controls to the causative agent is often rather unclear, case–control studies typically require study groups of considerable size.

When selecting candidate genes and gene variants for an association study it must be kept in mind that this selection is not always based on information on the gene and its product's roles, whereby exonic variation with structural amino acid substitutions may be attributed to the function of a protein, compared to variation located in intergenic or intronic chromosomal regions.

The candidate gene approach differs fundamentally from GWASs. In a GWAS, genetic variants covering the complete genome of an individual are scanned for common genetic variability without any *a priori* hypotheses. Notably, most genetic variation consists of single-nucleotide polymorphisms (SNPs), insertions and deletions. However, other types of variation, such as copy number variation (CNV) and others, may be subjected to genotyping as well.

A problem of candidate gene studies that do not confirm the hypothesis of a gene–phenotype association is that these studies are often not published, as publications of positive associations are more attractive. This publication bias can be overcome, but only if all sound attempts to establish

genetic associations with disease phenotypes, regardless of positive or lacking associations, are made available to the scientific community.

Here, we describe candidate genes that have been proposed to be involved in susceptibility to TB. The genes belong to the two large groups of receptors and cytokines/chemokines. For practical reasons, associations found in the major histocompatibility complex (MHC) region are addressed in the section entitled Receptors. A third set of genes, which do not belong to the receptors and cytokines/chemokines, will be addressed separately. Meta-analyses, where available, form an important basis of the compilation of candidate gene studies, as they bear the inherent advantage of a reasonable pre-selection of appropriate studies.

Receptors

CARD15

The nucleotide oligomerisation-binding domain 2 (NOD2) constitutes a pattern recognition receptor. Variability of the encoding gene, *CARD15*, has been shown to be associated with Crohn's disease. Recognition of mycobacterial components causes induction of cytokines to regulate pro-inflammatory responses. Several studies have investigated associations of *CARD15* variants with TB. No associations were found in Gambian and South African case–control groups [85, 86]. Weak associations with TB of three *CARD15* variants, P268S (rs2066842), R702W (rs139104022) and A725G (rs5743278) (OR 0.55 (95% CI 0.32–0.94) (p=0.02), OR 0.27 (95% CI 0.08–0.88) (p=0.01) and OR 2.16 (95% CI 1.01–4.72) (p=0.03), respectively) applied to African-Americans [56]. Associations described in a Chinese population [46] do not withstand statistical correction for multiple testing. *CARD15* Arg587Arg (rs1861759) has been claimed to be a risk factor for TB in the Chinese Han population [87].

CR1

Complement component receptor 1 (CR1) binds antigens opsonised by C3b and, thus, is involved in immune adherence and, eventually, in destruction of pathogens. Due to its function, *CR1* variants are promising candidates for TB association studies. Only one study so far has looked at associations in 514 cases and 913 controls from Malawi [88]. *CR1* Q1022H (rs56393589) homozygosity was associated with TB susceptibility (OR 3.12, 95% CI 1.13–8.60; p=0.028). This finding has not been rejected or validated.

DC-SIGN

The dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN, CD209) is a lectin on the surfaces of macrophages/monocytes. The molecule acts in cell adhesion and as a pathogen recognition receptor, and has been shown to be an important receptor for the cell wall mannose of *M. tuberculosis* [89].

Two promoter variants of the gene have been investigated, -336A/G (rs4804803) and -871G/A (rs735239). While earlier studies suggested positive genetic associations, a recent meta-analysis of 10 studies with 2,598 TB patients and 2,614 control individuals with differing ethnic backgrounds did not confirm an association of the -336 variant (OR 1.02, 95% CI 0.90–1.15) [90]. An association of the -871 variant as claimed to occur among South Africans [91] has never been confirmed in another ethnic group [92].

MARCO

Only one study so far has reported on an association of the macrophage receptor with collagenous structure (scavenger receptor class A), encoded by the *MARCO* gene [71]. *MARCO* is essential in the context of Toll-like receptor (TLR) signalling and, thus, in the response of macrophages once they encounter *M. tuberculosis*. A total of 923 Chinese Han TB patients and 1,033 healthy control individuals were genotyped for 17 tagging SNPs. rs17009726G turned out to be associated with increased susceptibility to TB (OR 1.65, 95% CI 1.32–2.05; p=0.00009). This finding has so far neither been corroborated by other studies nor contradicted.

MHC genes

The MHC encodes the human leukocyte antigen (HLA) molecules, which, based on their intriguing role in antigen presentation and the induction of cellular immune responses, are promising candidates for associations with infection phenotypes. Many studies on associations of factors of the HLA system with communicable and noncommunicable conditions have been conducted with conventional serological and modern molecular genetic techniques to date. A meta-analysis from 2006 included 22 studies on both class I and class II antigens with a total of 1,988 patients and 2,897 controls [53]. No associations of HLA class IA antigens were supported by the analysis, although strong differences in odds ratios were observed among the studies surveyed. The HLA class I allele B13 was, however, in the combined meta-analysis and in almost all original studies strongly associated with pulmonary TB (OR 0.64, $p<0.0001$). No associations were seen at the class I C locus, although a weak trend of association of the allele Cw2 with protection from TB was observed (OR 0.64, $p=0.08$). Among the HLA class II loci, DR3 and DR7 carriers were, to some degree, protected from TB (OR=0.72 ($p=0.002$) and OR 0.65 ($p<0.0001$), respectively). An increased risk of TB was observed among DR8-positive individuals (OR 1.72, $p=0.003$). The DR2 associations reported in several previous studies [93] were not consistently confirmed in the present meta-analysis. Interestingly, twin studies conducted in The Gambia have indicated that the impact of non-HLA genes significantly exceeds that of HLA genes [22].

P2X7

The purinergic receptor P2X7 is a ligand-gated cationic channel expressed in macrophages. Activation of the receptor contributes, after a cascade of intracellular events, to phagosome–lysosome fusion and to the death of mycobacteria. Associations of two genetic variants have been shown, with the variant 1513A/C (amino acid substitution of glutamic acid to alanine at codon 496; rs3751143) in exon 13 of the *P2X7* gene consistently identified and meta-analysed in seven studies in various populations [20]. The second variant, -762T/C (rs2393799) was found associated with protection from TB in a meta-analysis in two out of five studies in The Gambia and in Mexico, while no effect was seen in Russia. Enhanced susceptibility was observed in Han Chinese and Indian study groups [20].

TLR *genes and* TIRAP

Human TLRs participate as pattern recognition receptors in innate immune responses. They recognise pathogen-associated molecular patterns, and induce distinct genes to produce cytokines and other components of innate and, subsequently, acquired immunity.

Attempts to identify associations of several of the TLRs with the TB infection phenotype have been made. In particular, genes encoding TLRs 1, 2, 4, 6, 8 and 9 were studied. Studies of *TLR2* polymorphisms from Korea, Turkey and Tunisia [21, 65, 66] described the variants intron II microsatellite, rs5743708 and rs6265786 more frequently among TB patients than in controls. A more recent study [67] compared the occurrence of 71 *TLR1*, *TLR2*, *TLR4*, *TLR6* and *TLR9* variants in West-Africans, African-Americans and Caucasians (853 cases and 669 controls). The strongest association applying to West-Africans from Guinea-Bissau and to Caucasians were found for insertion/deletion polymorphisms (ins/del -196– -174; no rs number available) of *TLR2* (OR for Caucasians 0.41, 95% CI 0.24–0.68 ($p=0.0007$); OR for Africans 0.70, 95% CI 0.51–0.95; $p=0.023$). Associations of the *TLR9* polymorphisms rs5743836 and rs352143 in African-Americans and Caucasians were only marginally significant.

TB associations and the expression of 18 genes involved in the TLR pathways were studied in Indonesian (375 cases and 387 controls) and Russian study groups (1,837 patients and 1,779 controls). In a first step, four *TLR8* variants associated with TB were identified in the Indonesian group. When replicating this finding in the Russian group, rs3764880 was associated with TB among male patients only (OR 1.2, 95% CI 1.02–1.48; $p=0.03$) [68].

Several other studies on *TLR* associations with TB exist, with largely inconsistent results.

The TIR (Toll/interleukin (IL)-1 receptor) domain-containing adaptor protein MyD88 (TIRAP) is involved in the TLR signal cascade. A first study on *TIRAP* variability was conducted in a Vietnamese study group, where the C558T *TIRAP* polymorphism (rs7932766) was associated with TB [69]. In a study of several infection phenotypes among 6,106 individuals (in the UK, Vietnam and Africa), *TIRAP* rs8177374 was associated with protection from invasive pneumococcal disease, bacteraemia, malaria and TB [94]. This finding was rejected for TB in a combined replication analysis of Russian, Ghanaian and Indonesian populations [95], and also by a recent meta-analysis [96].

TNFRSF1B

A study of independent case–control populations from South Africa and a fraction of the aforementioned study group from Ghana investigated associations of four polymorphisms of the *TNFRSF1B* gene (tumour necrosis factor (TNF) receptor 2-encoding gene, TNF receptor superfamily member 1B). The receptor occurs on several cell types, particularly on the surfaces of myeloid cells, and on activated circulating T- and B-lymphocytes. In addition, it is strongly involved in the regulation of apoptosis in CD8+ cells.

The South African study group consisted of 429 and 482 and the Ghanaian group of 640 and 1,158 cases and control subjects, respectively [63]. In South Africans, an association in the 3′-untranslated region (UTR) of *TNFRSF1B* was detected. The rs3397T allele and/or the 3′-UTR haplotype GTT (rs1061624G, rs5030792T and rs3397T) conferred protection against TB; however, this applied only to females. It was replicated in Ghanaian female case–control individuals, providing similar odds ratios of 1.3 in both study populations and a significance level in the combined analysis of $p=0.0037$.

VDR

Deficiency of the vitamin D receptor (VDR) has been shown to contribute to increased TB susceptibility [94, 97]. Notably, TB has, for generations, been treated with vitamin D substitution. Addition of vitamin D to suspensions of infected macrophages increased successful elimination of *M. tuberculosis in vitro*, but the responsible mechanisms of the eliminating process have not yet been clarified [47, 96, 98]. In the search for genetic variants associated with susceptibility to TB, most studies comprised analyses of four *VDR* variants, addressed as *Fok*I (rs10735810), *Taq*I (rs731236), *Bsm*I (rs1544410) and *Apa*I (rs7975232). In a meta-analysis of 23 studies [48], inconsistent results, depending on the population under survey, were obtained. Significant associations with susceptibility were observed in studies of Asian populations for the ff-*Fok*I genotype (OR 2.0, 95% CI 1.3–3.2) and with protection for the bb-*Bsm*I genotype (OR 0.5, 95% CI 0.4–0.8). In addition, weak associations were observed among Asians for the *Taq*I variant (OR 1.4, 95% CI 0.9–2.1). The association of the aa-*Apa*I genotype in African populations awaits verification. No further associations were observed in African and South-American populations.

Cytokines and chemokines

CXCL10

The chemokine CXC motif ligand (CXCL)10 is involved in regulation of leukocyte trafficking. One study so far (240 Chinese patients and 176 controls only) investigated associations of *CXCL10* variants with TB [99]. A discrete association of the promoter variant -135G/A (rs56061981) with TB was observed (OR 0.51, 95% CI 0.29–0.91; $p=0.01$). The finding awaits replication.

CTLA4

Cytotoxic lymphocyte-associated antigen (CTLA)4 (CD152) belongs to the immunoglobulin superfamily and is expressed by activated T-cells. It binds to CD80 and CD86 on antigen-presenting cells. CTLA4 is a negative regulator of T-cells. The observation that T-cell proliferation and antibody responses were reduced among TB patients carrying the *CTLA4* +49G (rs231775) allele made *CTLA4* a candidate for TB case–control studies [16]. The frequencies of *CTLA4* +6230A/G (rs3087243) and six haplotype-tagging SNPs were compared in a Ghanaian study group

of 2,010 TB patients and 2,346 controls. No differences in the frequencies of variants were observed between patients and controls. However, the variant +6230A and a distinct *CTLA4* haplotype occurred significantly less frequently among cases with extended opacities in chest radiographs compared with those with less prominent lesions (p=0.00045) [100].

IL1

The pro-inflammatory chemokine IL-1β and the corresponding inhibitor IL-1 receptor antagonist (IL-1Ra) are induced by *M. tuberculosis*, as shown in *in vitro* experiments. In addition, IL-1Ra was seen to influence TB-disease activity and a study has shown that TB patients have elevated levels of IL-1Ra [39]. A small case–control study of 89 Indian TB patients and 114 healthy controls examined a haplotype consisting of the *IL1Ra* microsatellite allele (A2) and the *IL1b* +3953 (rs1143634) (A1) variant. The haplotype was more frequent among TB patients with pleurisy than among healthy controls (p=0.028) [40]. Further studies of *IL1b* gene variants showed protection associated with both heterozygosity and homozygosity of the *IL1* -511C promoter allele (rs16944) in The Gambia (OR 0.66 (p=0.027) and OR 0.58 (p=0.015), respectively) [41] and with the *IL1B* +3953T allele in Colombia (OR 0.3, 95% CI 0.1–0.6; p=0.001) [101]. The association could not be confirmed in either Cambodia or Peru [57, 58].

IL8

IL-8 is a chemokine involved in inflammation, granuloma formation and chemoattraction of leukocytes to sites of inflammatory events. An association study performed in Caucasian and African-American TB patients and controls revealed an association of the promoter *IL8* -251A allele (rs4073) occurring homozygously with clinical TB (OR for Causcasian Americans 3.41, 95% CI 1.52–7.64 ($p \leqslant 0.01$); OR for African-Americans 3.46, 95% CI 1.48–8.08 ($p \leqslant 0.01$)) [59]. This finding could not be replicated in a larger cohort from The Gambia [60].

IL10

IL-10 is secreted by T-helper cell (Th) type 2 lymphocytes and monocytes. IL-10 participates in an anti-inflammatory manner in the regulation of the immune response. In particular, it inhibits cytokines involved in the Th1 response. A variety of studies have looked at association of TB with variants of the encoding gene, *IL10*. Of particular interest are three polymorphisms in the *IL10* promoter region, -1082G/A (rs1800896), -819C/T (rs1800871) and -592A/C (rs1800872). A recent meta-analysis [61] has re-analysed 18 case–control studies with varying numbers of study participants, depending on the polymorphisms analysed (4,740 cases and 5,919 controls for the *IL10* -1082 polymorphism, 2,696 cases and 3,935 controls for the *IL10* -819 variant, and 3,070 cases and 4,596 controls for the *IL10* -592 variant). The combined analysis did not yield significant results for any of the variants. However, when stratifying for ethnic groups, the comparison of the -1082 genotypes AA and AG *versus* the GG genotype revealed relative protection among Europeans conferred by the AA/AG genotypes (OR 0.55, 95% CI 0.35–0.88; p=0.01). Although the Ghanaian study did not show significant differences of *IL10* alleles in the case–control comparison, the IL-10 low-producer haplotype *IL10* -2849A (rs6703630)/-1082A/-819C/-592C, compared with the high-producer haplotype -2849G/-1082G/-819C/-592C, was observed less frequently among purified protein derivative (PPD)-negative controls than among cases (OR 2.15, 95% CI 1.3–3.6; p=0.013) and PPD-positive controls (OR 2.09, 95% CI 1.2–3.5; p=0.017) [62]. Lower IL-10 plasma levels in homozygous -2849A/-1082A/-819C/-592C carriers were in that study confirmed by an IL-10 ELISA (p=0.016).

Interferon-γ pathway

IFNG

Interferon (IFN)-γ is an important cytokine of innate and acquired immune responses agai*nst M. tuberculosis* and other pathogens. It has important effects in the stimulation and modulation of immune responses. IFN-γ is produced by natural killer (NK), CD4 and CD8 positive T-lymphocytes. A recent meta-analysis of the *IFNG* +874 variant (rs2430561) in 19 TB studies with a

total of 4,752 cases and 4,935 controls found the TT genotype associated with protection (OR 0.77, 95% CI 0.67–0.88), whereas the AA genotype was associated with susceptibility (OR 1.51, 95% CI 1.38–1.65), applying a random effects model accounting for heterogeneity in the study groups [102]. In a case–control study from The Gambia, the *IFNG* promoter variant -1616GG (rs2069705) and the variant +3234TT (rs2069718) were found to be associated with susceptibility (OR 1.49 (95% CI 1.11–2.00) (p=0.008) and OR 1.40 (95% CI 1.09–1.80) (p=0.009), respectively) [49].

IFNGR1

The IFN-γ receptor consists of IFNGR1 and IFNGR2. IFNGR1 binds IFN-γ. A study of *IFNGR1* variants in the context of TB susceptibility yielded a significant protective effect of an intronic (CA)n microsatellite (95% CI 0.14–0.94, p=0.02) [50]. This could not be replicated in the Gambian TB study group [103]. Another study of this microsatellite in an Indonesian case–control group described an association with the CA_{12}/CA_{12} genotype (OR 0.5, p=0.01) [51].

In the Gambian group, an association with TB was found with the *IFNGR1* promoter -56CC genotype (rs2234711) (OR 0.75, 95% CI 0.57–0.99; p=0.041) [49].

IFNGR2

When examining 832 TB patients and 506 controls from Vietnam, significant associations were identified with a microsatellite marker in the 5′-upstream region of the *IFNGR2* gene (p=0.036) and the rs2834213GG genotype (OR 0.70, 95% CI 0.57–0.86; p=0.00054) [52].

IL12A

IL-12A (p35) is a subunit of IL-12. It acts mainly in the activation of innate and adaptive immune responses. After the identification of *IL12A* as a candidate gene in a genome-wide linkage study [81], the *IL12A* genotypes TG/GG of rs2243115 were found in 522 Chinese TB cases and in 527 controls associated with a decreased risk of TB (OR 0.67, 95% CI 0.48–0.94; p=0.021) [54].

IL12B

IL-12B (p40) is the second component of IL-12. In a study of a Hong Kong Chinese case–control group a microsatellite marker, $(ATT)_8$, of the *IL12B* gene was associated with TB susceptibility (OR 2.14, 95% CI 1.45–3.19; $p \leqslant 0.001$) [55]. Nine *IL12B* polymorphisms were tested for associations in participants from The Gambia, Guinea-Bissau, African–Americans and Argentina. No consistent results could be observed when combining the results of all four countries [42].

IL12RB1

IL-12RB1 is part of the receptor for IL-12. A haplotype consisting of three *IL12RB1* missense variants (R214-T365-R378) (rs11575934, rs375947 and rs401502) was reported to be associated with increased susceptibility to TB in a Japanese TB case–control study (OR 2.45, 95% CI 1.20–4.99; p=0.013) [104]. This association could not be confirmed in two studies with participants from Morocco and Korea. Two *IL12RB1* promoter variants (-2 and -111; rs436857 and rs393548) were, however, correlated with disease in the Moroccan study (OR 2.03 (95% CI 1.04–4.04) (p=0.019) and OR 2.69 (95% CI 1.19–6.09) (p=0.013), respectively) [43].

A small study in a Japanese case–control group (87 cases *versus* 265 controls) revealed associations of the *IL12RB1* variants 641A/G, 1094T/C and 1132C/G, corresponding to the amino acid positions 214, 365 and 378, with clinical TB [17].

With regard to other genes involved in the IFN-γ pathway, no consistent associations have so far been observed.

MCP1

The gene encoding monocyte chemotactic protein-1 (*MCP1*) belongs to the family of small inducible genes. Its product is involved in the recruitment of monocytes at relevant sites of inflammation and infection. Genetic association studies of *MCP1* polymorphisms have so far led

to inconclusive findings, depending on the ethnic group in which variants were studied. A meta-analysis [19], and a recent case–control study from West Africa, Argentina and the USA summarise the current knowledge [105]. The meta-analysis comprised 4,676 tuberculosis cases and 5,260 controls from Ghana, China, India, Korea, Peru, South Africa and Mexico. In a combined analysis of all ethnic groups, the -2581G variant (rs1024611) was associated with susceptibility to TB (OR 1.51, 95% CI 1.11–2.04; p=0.008). When stratifying for the ethnic groups of Asians, Latin Americans and Africans, the -2581G allele was associated with susceptibility in Asians and Latin Americans, whereas, more credibly and with a far more convincing statistical significance, in Ghanaians and South Africans, it was found to be associated with relative protection from TB. The finding among Africans is largely due to the study of Ghanaian participants [18], as this study group comprises more than 50% of the individuals in the meta-analysis. In the Ghanaian study and that from West Africa, Argentina and the USA, an additional variant, -362 (rs2857656), was analysed. While in the Ghanaian study a protective effect was attributed to the -362C allele (OR 0.83, 95% CI 0.76–0.90; p=0.00017), no association was observed in the groups from West Africa (populations other than Ghanaians), Argentina and the USA.

In addition to the earlier results obtained in the Ghanaian TB case–control sample, a haplotype containing the combination -2581G/-362C/int1del554–567 was found to mediate considerably stronger protection than did the *MCP1* -362C allele alone (OR 0.78 (95% CI 0.69–0.87) *versus* OR 0.83 (95% CI 0.76–0.91), respectively) [44]. The findings indicated a largely negligible role of the variant at position -2581 in the Ghanaian population studied.

TNF

The gene encoding TNF-α, *TNF*, is located in the class III region of the MHC. TNF is a pro-inflammatory cytokine with a multitude of effects, *e.g.* in metabolic processes, endothelial functions, coagulation and others. In TB, TNF is involved in granuloma formation. In clinically advanced TB, significantly elevated serum levels of TNF-α may be found when compared with mild cases of TB and controls [106]. Many case–control association studies of a large number of diseases, including malaria, AIDS, psoriasis and others, have been published to date. The polymorphism genotyped in most conditions is the *TNF* promoter variant -308G/A (rs1800629). A meta-analysis of *TNF* -308 associations in TB recently analysed 18 studies including a total of 2,584 cases and 3,817 controls [45]. In the combined groups, no significant association was observed. However, when stratifying for the two ethnic groups of Caucasians and Asians, and analysing genotype and allele frequencies, results were inconsistent, with two marginal associations observed in the subgroups [32–34, 107].

With the exception of a marginal association of the -308A/-238G (rs1800629, rs361525) *TNF* haplotype among Colombian TB patients [32], no convincing associations were observed when genotyping variants other than the *TNF* -308 polymorphism.

Other genes

ALOX5

The 5-lipoxygenase (ALOX5)-derived lipid mediators regulate inflammation by adjusting activities of immune cells and the production of cytokines. Host control of *M. tuberculosis* is regulated by ALOX5, as demonstrated in *ALOX5*-deficient mice [108]. Variable numbers of tandem repeats (VNTRs) of the *ALOX5* promoter and the exonic nonsynonymous variant g.760G>A were analysed by microsatellite determination and fluorescence resonance energy transfer, respectively, and DNA sequencing in 1,916 TB patients from Ghana and in 2,269 healthy controls. In addition, mycobacterial lineages of more than 1,400 isolates were differentiated. Carriers of one variant (number of repeats not equal to 5) and one wild-type VNTR allele or of the allele g.760G/A (rs2228065) had an increased risk of TB (OR 1.19 (95% CI 1.04–1.37) (p=0.026) and OR 1.21 (95% CI 1.04–1.41) (p=0.026), respectively). The g.760G/A association was stronger in TB caused by the clade *M. africanum* West-African 2 [109]. The *ALOX5* association with TB underlines the role of

ALOX5 products in regulating immune responses to *M. tuberculosis*. This was one of the first studies that analysed the interplay of host and pathogen genetic factors in a case–control design.

IRGM

Another example of the interplay of host and pathogen genetic factors is provided in an association study of the human immunity-related GTPase M (IRGM), which has an important role in the control of autophagy [70]. *IRGM* gene variants were studied in 2,010 Ghanaian TB patients and in 2,346 unaffected controls [110]. Mycobacterial clades were classified by spoligotyping, *IS6110* fingerprinting and genotyping of the *pks*1/15 deletion. The *IRGM* genotype -261TT (rs9637876) was associated with resistance to TB when the infection was caused by *M. tuberculosis* (OR 0.66, 95% CI 0.52–0.84; p=0.0045), but not with resistance from infections caused by *M. africanum* or *Mycobacterium bovis* (OR 0.95, 95% CI 0.70–1.30; p=0.8). More detailed molecular analyses of clades and stratification for mycobacterial lineages revealed that protection applied only to infections with *M. tuberculosis* strains with defects of the *pks*1/15 gene, which is characteristic for the Euro-American (EUAM) subgroup of *M. tuberculosis* (OR 0.63, 95% CI 0.49–0.81; p=0.0019). Together with a luciferase reporter gene assay including the *IRGM* variants -261C and -261T, an important role of IRGM and autophagy in protection against natural infection with *M. tuberculosis* EUAM was strongly suggested [110].

Another case–control study in China investigated a 1.7-kb promoter region of *IRGM1* and identified the -1208AA genotype (rs4958842) (OR 0.58, 95% CI 0.34–0.98; p=0.042) to be associated with susceptibility to TB [111]. The most recent study of *IRGM* examined the variant rs10065172C/T, which previously has been found to be associated with Crohn's disease. A total of 370 African-American and 177 Caucasian TB patients were compared with 180 and 110 healthy control individuals, respectively. African-American TB patients were more likely to carry the rs10065172 T allele (OR 1.54, 95% CI 1.17–2.02; p=0.01) compared with controls [112]. Notably, the variant found to be associated in this study did not show any effect, even not as a trend, in the larger study from Ghana.

MBL2

Mannose-binding lectin (MBL) is a plasma opsonin and belongs to the collectin family. It forms homotrimers that associate to form higher oligomers, preferentially hexamers. MBL plays an important role in immune responses, in particular, at first contacts with microbial pathogens. It binds to carbohydrates on the surfaces of many of microorganisms and promotes phagocytosis directly through a yet undefined receptor or indirectly *via* activation of the complement lectin pathway. MBL2 contributes also to other pathways of inflammation and plays a role in vascular and autoimmune disease as well as in apoptosis. Structural variants of *MBL2* cause quantitative and qualitative functional deficiencies, which are associated with various patterns of susceptibility to infectious diseases and other disorders.

Several *MBL2* polymorphisms have been included in TB association studies, in particular, variants at codons 52, 54 and 57 of *MBL2* exon 1 (rs5030737, rs1800450 and rs1800451). In a meta-analysis, 12 trials with a total of 1,815 patients *versus* 2,666 controls were re-analysed and compared [113]. While serum MBL levels were consistently elevated in TB, no significant associations of genotypes with susceptibility transpired in the combined analysis. A drawback of the meta-analysis was the different study designs and their heterogeneity. The meta-analysis clearly stated that the conclusions drawn are not applicable to all subpopulations. In four out of the 17 studies, additional promoter variants were genotyped without any significant association.

In a more recent study of the Ghanaian study group (2,010 TB patients, 2,346 control individuals), a protective association between susceptibility to TB and the *MBL2* G57E variant (OR 0.60, 95% CI 0.4–0.9; p=0.008) was found when assuming a recessive mode of inheritance [10]. Interestingly, this association applied only to infections caused by *M. africanum*, as confirmed by functional binding studies. *M. africanum* isolates bound recombinant human MBL *in vitro* more efficiently than did isolates of *M. tuberculosis sensu stricto*, suggesting that TB might have

favoured the spread of MBL deficiencies and the causing polymorphisms in African regions where *M. africanum* exclusively occurs. These observations again underline the value of pathogen genotyping and relating pathogen to host variability in association studies.

NOS2A

After the identification, by linkage studies, of an association between the gene encoding nitric oxide synthase 2 (*NOS2A*) and TB susceptibility [114], a study of 114 TB patients and 304 controls from Colombia looked at a polymorphic (CCTTT)n microsatellite and a TAAA promoter insertion/deletion (rs numbers not available). While individuals carrying short *NOS2A* CCTTT alleles (8–11 repeats) were relatively protected from TB (OR 0.63, 95% CI 0.46–0.86; p=0.005), no associations were found for the ins/del TAAA polymorphism [31].

The promoter variants rs2779249 and rs2301369 (*NOS2A* -1026 and -2447) were associated with tuberculosis (p=0.039 and p=0.029, respectively) in 92 families in Brazil [29], while rs1800482 (*NOS2A* -954) was not correlated with TB in Mexico [30].

A larger TB case–control group was studied in South Africa and a haplotypic association, with the functional rs9282799 and rs8078340 variants, was identified [115]. While rs8078340T was more frequent among cases (OR 1.4, 95% CI 1.1–1.8; p=0.038), rs8078340C was overrepresented in controls (OR 1.4, 95% CI 1.1–1.8; p=0.029). 10 variants were nominally associated with TB in African-Americans, but only two of them passed the correction threshold for multiple testing (OR for rs2274894 1.84, 95% CI 1.23–2.77 (p=0.003); OR for rs7215373 1.67, 95% CI 1.17–2.37 (p=0.004)) [116]. The associations described for *NOS2A* need to be replicated and confirmed in other study groups and other ethnicities.

NRAMP1 *(SLC11A1)*

After the identification of the mouse *Nramp1* gene and a correlation of *Nramp1* variability with susceptibility to mycobacteria (bacille Calmette–Guérin; BCG) [23], the human homologue, *NRAMP1* (*SLC11A1*) was the first candidate gene to be investigated in TB. *NRAMP1* encodes natural resistance-associated macrophage protein 1 (NRAMP1), also known as solute carrier family 11 (SLC11A1). The gene product has several functions, which are related to activation of macrophages, the function of neutrophils and to innate immune responses. Numerous groups have tried to confirm the first report on *SLC11A1* polymorphisms and their association with susceptibility to TB [24]. A systematic review and a meta-analysis on *SLC11A1* in TB analysed results of 36 studies and summarised the quantitatively observed associations. The meta-analysis concluded that *SLC11A1* polymorphisms might have a role in TB susceptibility [25]. Four distinct variants were looked at in detail, namely the 3′-UTR variant (rs17235416), the polymorphism D543N (rs17235409), INT4 (rs3731865) and the 5′-(GT)n variant (rs17235416). Odds ratios were between 1.35 and 1.23, indeed arguing that these are susceptibility alleles. When stratifying for the different ethnic populations of Africans, Asians and Caucasians, associations were observed for at least one of the four allelic variants. Importantly, the sample size of most of the study groups was rather small and insufficient to meet standard requirements of modern genetic epidemiological studies.

PTPN22

Tyrosine phosphatase nonreceptor type 22 (*PTPN22*) is a phosphatase specific to lymphoid cells, and is involved in inflammatory responses and T-cell activation. A first study of *PTPN22* in a Colombian group with 113 patients and 161 controls reported on an association of the R620W variant (rs2476601) with protection from TB (OR 0.3, 95% CI 0.08–1.04; p=0.04) [26]. Another study, again with 123 cases and 155 controls only from Morocco confirmed that finding (OR 0.14, 95% CI 0.01–0.93; p=0.01) and described also an association of the R263Q variant with susceptibility (OR 5.85, 95% CI 1.17–39.55; p=0.01) [27].

SP110

Studies of *SP110* were based on the function of the nuclear body protein SP110, which belongs to the SP100/SP140 family and appears to activate gene transcription. The protein is also involved in

apoptosis-related processes, in control of the cell cycle and in immune responses. The mouse homologue of *SP110*, intracellular pathogen resistance 1 (*Ipr1*), appears to control innate immune responses, and limit replication of *M. tuberculosis* and several other pathogens [28].

The first study that reported on associations of the rs2114592 and rs3948464 variants was performed in The Gambia in 219 families, and then replicated in 99 and 102 families from Guinea and Guinea-Bissau, respectively [35]. At the same time, a lack of *SP110* association was reported the Ghanaian study group [36], and clearly confirmed in subsequent studies from South Africa, Russia, India and Indonesia [37, 38, 117, 118].

Genome-wide association studies

GWASs have recently become feasible through substantial methodological progress. This type of study has largely replaced linkage studies, which were invaluable tools for the identification of monogenic causes of disease phenotypes, but often failed in the analysis of the underlying causes of polygenic, complex diseases. In contrast to case–control studies, GWASs allow the identification of genetic regions of interest in a hypothesis-free manner, and provide insight into *a priori* unrecognised pathophysiological mechanisms, biochemical pathways and immunological properties of a disease phenotype. As a consequence of the large number of independent SNPs genotyped and the inherent need of multiple testing (Bonferroni correction), a GWAS group must be of significant size in order to obtain robust results and to reliably indicate a disease locus. Depending on the genotyping platform applied, GWASs can determine millions of common genetic variants across the entire genome simultaneously (approximately 1 million SNPs or more) and, in one of the GWAS systems, a large number of CNVs. The number of SNPs may again be increased by custom made genotyping products. Results are given, as in candidate gene studies, as odds ratios. As in candidate gene studies, a GWAS design mostly involves case–control study groups. However, in most cases, assignations of causality are difficult, if not impossible, to make. Many diseases have been investigated in GWASs, with more than 4,000 SNPs found to be associated with many phenotypes.

A major advent and a prerequisite for GWASs is the ongoing maintenance and service provided by the public access to several electronic repositories of human genetic data, such as dbSNP [119], HapMap [120], the 1000 Genomes Project [121] and others. Statistical analyses are typically performed using software tools such as PLINK [122] or SNPTEST [123]. Limitations of GWASs may arise from insufficient quality control of primary results, insufficient phenotyping and a lack of correction for multiple testing, an insufficient size of the study groups under survey, and lack of stratification for confounders such as age, sex and ethnicity. In principle, most of these limitations have been adequately addressed and standards established [124]. Other genome-wide methodologies, such as whole-exome sequencing, have, in the resolution of genetic causes of TB, not yet been applied.

A first successful attempt to identify novel genetic associations in a TB case–control study group was made in the large case–control group from Ghana [72]. The Affymetrix SNP array 6.0 was used in that study. After weakly significant results in that GWAS, results obtained in another study group from The Gambia after application of the Affymetrix 500K array were combined with the results from the Ghana group. When replicating the findings in additional Ghanaian and Malawian TB patients and controls (a total of 11,425 individuals), variant rs4331426G in a gene-poor region on chromosome 18q11.2 was found to be associated with TB (OR 1.19, 95% CI 1.13–1.27; combined $p=6.8 \times 10^{-9}$). The high degree of conservation of this region suggests that it is involved in regulatory processes of gene expression. This first GWAS on TB clearly demonstrated the capacity of GWASs to identify new susceptibility loci for infectious diseases, even in the highly genetically diverse African populations.

After imputation of data made available through the 1000 Genomes Project into the genome-wide dataset of the Ghanaian TB case–control group, a resistance locus on chromosome 11p13

downstream of the *WT1* gene was identified [73]. The strongest signal was obtained with the rs2057178A allele ($p=2.63\times10^{-9}$). The replication in the Gambian, Indonesian and Russian TB case–control groups (total number of individuals included 22,680) increased the significance of this association to $p=2.57\times10^{-11}$. *WT1* encodes a DNA-binding protein that functions as a transcriptional element. The protein is required for the unimpaired development of the genitourinary system and parts of the mesothelium.

A subsequent GWAS of a far smaller group of Thai and Japanese TB case–control groups did not yield conclusive results. A risk locus in an intergenic region of chromosome 20q12 was found only after stratification for the age of participants [74].

In a study of an Indonesian case–control cohort and an attempt to identify additional genetic targets, a small sample of an Indonesian case–control group was genotyped with the low-resolution Affymetrix 100K SNP assay (95,000 SNPs). Validation was attempted in larger cohorts, including a representative sample from Russia [75]. Suggestive associations with p-values of 0.0004–0.006 for eight independent loci, located within or close to the genes *JAG1* (Jagged 1), *DYNLRB2* (dynein, light chain, roadblock type, 2), *EBF1* (early RLY B-cell factor 1), *TMEFF2* (transmembrane protein with epidermal growth factor-like and two follistatin-like domains 2), *CCL17* (chemokine, CC motif, ligand 17), *HAUS6* (HAUS augmin-like complex, subunit 6), *PENK* (proenkephalin) and *TXNDC4* (thioredoxin domain-containing protein 4), were observed [75]. These findings await replication and confirmation, including fine-mapping of the eight potential candidate genes.

Conclusion

The overall aim of genetic association studies is to identify targets for intervention or prevention of a disease. Although a wealth of genetic studies in TB has been conducted to date, none of the genetic associations in TB has so far led to the development of promising tools for prevention or treatment. The reasons are manifold and mostly comprise either flaws in the study design or a lack of functional evaluations of associations. First, associations of human host genetic factors with susceptibility to TB observed are mostly inconclusive across reports and study populations. Secondly, and most importantly, many studies did not have sufficient statistical power to provide truly significant results. This is, in most cases, a result of study group sizes, which are often too small, but also often due to a lack of stringent statistical requirements. Meaningful results cannot be obtained when comparing a small number of patients with an equally small number controls. In contrast to monogenic diseases, where one gene exerts strong effects, the analysis of polygenic conditions is far more challenging. It is worth noting that, in polygenic diseases, even low odds ratios of, for example, 1.2 or even lower may indicate molecules or pathways that merit further investigation.

The association studies performed with the Ghanaian study group of 2,010 patients and 2,346 (in the GWAS, >5,500) healthy controls, supplemented in the GWAS with large case–control groups from The Gambia, Malawi, Indonesia and Russia, provide examples of a design that provides sufficient power. Only with the size of that study and large groups available for replication could convincing results be obtained in the GWAS [72, 73, 125]. Although the signals were identified in "gene deserts", the significance clearly requires further investigation, in particular as gene influences are not necessarily subjected to *cis*-regulation but may occur in a *trans*-acting manner.

Much is to be done, including sound fine mapping of associated chromosomal regions and in-depth utilisation of new and developing techniques and tools to further study gene–gene interactions and pharmacogenomics [116, 126, 127], pathogen genetic factors and their relationship with distinct host genotypes, and chemical processes of metabolites and their effects on cellular activities. These efforts must be supplemented by large-scale assessment of structures and functions of proteins, and functional variation of proteins in health and disease.

Statement of Interest

None declared.

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Chapter 5

State of the art in vaccine development against TB

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SUMMARY: Each year, more than 1.5 million people die of tuberculosis (TB) and over 9 million individuals newly develop active disease. The currently available tools are inadequate to control infection and combat TB. The only TB vaccine currently in use, *Mycobacterium bovis* bacille Calmette–Guérin (BCG), affords incomplete and highly variable protection against TB, and better TB vaccines are urgently needed. Here we discuss recent progress in developing, testing and clinically evaluating new TB vaccines. A dozen new TB vaccine candidates have been and are being evaluated in human clinical trials. Future perspectives and directions for new TB vaccines will also be discussed.

KEYWORDS: Biomarkers, innate and adaptive immunity, live vaccines, subunit vaccines, tuberculosis

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The bacterium *Mycobacterium tuberculosis* is an intracellular pathogen that is currently responsible for more human deaths today than any other single pathogen today [1–3]. Over 1.5 million people die of tuberculosis (TB) a year and more than 9 million newly develop active TB annually [4]. Over 2 billion people are thought to be latently infected with *M. tuberculosis*. Between 3% and 10% of all latently TB-infected individuals will develop active TB disease during their lifetime. Co-infection with HIV dramatically enhances susceptibility to TB, and increases TB reactivation rates from 3–10% per lifetime to 5–10% per life-year [5]. The increasing prevalence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *M. tuberculosis* strains [6], and the appearance of totally drug-resistant (TDR) *M. tuberculosis* strains [7] further complicate the control of TB with conventional tools.

The only TB vaccine registered today for use in humans is *Mycobacterium bovis* bacille Calmette–Guérin (BCG). BCG vaccination is widely used and significantly protects infants against severe TB, in particular TB meningitis, which has a very high case fatality rate. However, BCG vaccination protects inconsistently and incompletely against pulmonary TB in adults, which is responsible for TB transmission. Moreover, a more recent concern is that HIV-infected infants are at an increased risk of developing disseminating "BCGosis" [8] due to their immunodeficiency. This complication is also found in individuals with rare genetic deficiencies in the interleukin (IL)-12/IL-23/interferon (IFN)-γ/signal transducer and activator of transcription (STAT)1 pathway [9, 10]. Thus, besides developing better TB vaccines, safer vaccines than the BCG are also needed [8].

In the last two decades, significant progress has been made in TB vaccine research, in preclinical testing of such vaccines and, more recently, in phase I/II clinical testing in humans. More than 12 novel TB vaccine candidates have been or are being tested for safety and immunogenicity in clinical trials.

At the same time, new approaches are being considered to design further improved TB vaccines with superior safety, immunogenicity and protective efficacy profiles when compared with the BCG vaccine.

Status of the current *M. bovis* BCG TB vaccine

The BCG vaccine is one of the most widely administered vaccines today, and has been given over 4 billion times. Since the 1970s, it has been part of the Expanded Program on Immunization (EPI). Although BCG is a live vaccine, it is very safe with only very few adverse events reported. However, despite its impressive safety record, recent data indicate that BCG can cause disseminating BCGosis in immunocompromised individuals, due to genetic defects in host defence or due to HIV infection, particularly in infants (as previously discussed). BCG can, therefore, pose a risk to infants in HIV-burdened areas [8]. The World Health Organization (WHO) Global Advisory Committee on Vaccine Safety has, therefore, decided to advise against using BCG in HIV-positive children. Another concern regarding BCG is its limited efficacy against TB. New TB vaccines are needed to either boost BCG to achieve improved infection control or, alternatively, replace BCG as more effective priming vaccines with improved safety profiles. These two strategies can be combined in combinatorial regimens. After a short introduction into the immunology of TB, these new types of TB vaccines will be discussed.

Host defence to *M. tuberculosis* infection

Infection with *M. tuberculosis* is contained in the vast majority of infected individuals. Only 3–10% of those infected will develop active TB disease during their lifetime, mostly in the first 2 years following infection, while the remainder will develop latent TB infection (LTBI) [11]. LTBI is classically measured by a conversion of the tuberculin skin test (TST). This test measures classical delayed-type hypersensitivity reactivity to purified protein derivative, a soluble extract of *M. tuberculosis* antigens that is injected intradermally. Based on TST surveys, one-third of the world's population is estimated to be infected with *M. tuberculosis* [4]. This large number of LTBI cases gives rise to the high numbers of newly developed active TB cases [12]. Besides the TST, diagnostic tests that distinguish more specifically between *M. tuberculosis* infection and BCG vaccination or infection with non-tuberculous mycobacteria (NTM) have recently been developed. These assays measure *M. tuberculosis* antigen-specific release of IFN-γ in whole-blood assays or ELISPOT assays, and are best known as IFN-γ release assays (IGRAs).

Granuloma formation

Upon inhalation, *M. tuberculosis* aerosols reach the alveoli, where *M. tuberculosis* subsequently infects alveolar macrophages, dendritic cells (DCs) and perhaps also other cells, such as epithelial cells (fig. 1). Other cells start being recruited to this initial infection focus, including macrophages, neutrophils and DCs. This leads to the formation of a cellular infiltrate, which becomes organised as a primary granuloma. *M. tuberculosis* is able to persist inside host cells and evade antimicrobial defense mechanisms. *M. tuberculosis*, for example, impairs phagosome maturation, autophagy, apoptosis, major histocompatibility complex (MHC) antigen processing and presentation and IFN-γ receptor signalling. Moreover, *M. tuberculosis* can escape from the phagosomes into the cytosol. Each of these mechanisms help *M. tuberculosis* to evade innate antimicrobial host defences [13–16]. In the next phase of the infection, *M. tuberculosis* also evades the ensuing adaptive immune response. *M. tuberculosis* has a remarkable ability to delay the initiation of adaptive immune responses when compared with other pathogens, both in humans [17] and in mice [18–22]. This allows *M. tuberculosis* to establish primary infection and reach a critical mass of bacilli to propagate infection. *M. tuberculosis* bacilli that are not eliminated immediately by innate host defence mechanisms can enter a "dormant" or "nonreplicating" phase in which they can persist in a metabolically inactive state inside infected cells. These bacteria can "reactivate" many years later, resume active replication and cause active TB disease [23].

Balanced inflammation

As soon as the adaptive immune response has been induced, T-cells are recruited to the granuloma. This results in the formation of a mature, solid and well-organised granuloma that will contain

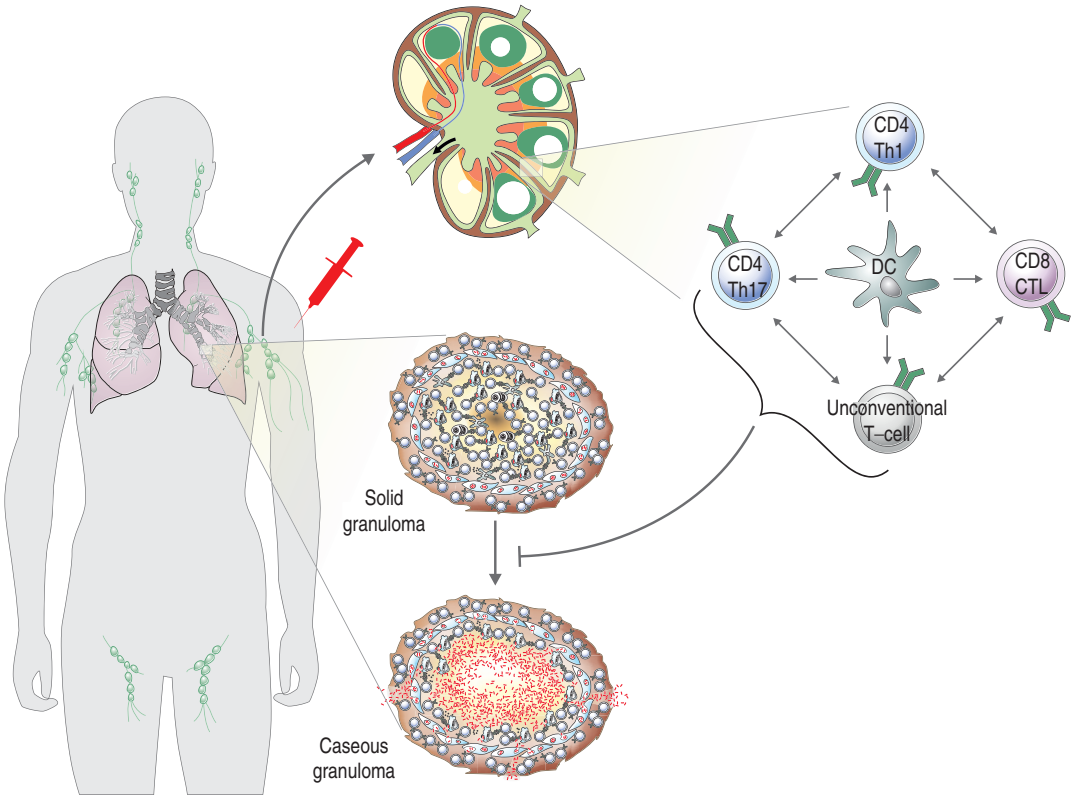

Figure 1. Efficacious vaccination against tuberculosis (TB) depends on a fine-tuned balance between different T-cell populations. The figure schematises the development of TB from latent TB infection, characterised by solid granulomas that contain *Mycobacterium tuberculosis*, to active TB disease, characterised by caseous granulomas that cause damage and allow dissemination of *M. tuberculosis*. Vaccination stimulates a fine-tuned balance composed of different T-cell populations in draining lymph nodes, including: CD4+ T-helper (Th) type 1 cells as the main mediators of protection; CD4+ Th17 cells, which could play a role in early stages of protective immunity; CD8+ T-cells with cytolytic T-lymphocyte (CTL) activity, which also produce Th1-like cytokines and contribute to protection; and unconventional T-cells with similar phenotypes and functions. DC: dendritic cell.

M. tuberculosis organisms. However, balanced inflammation remains important in TB: if inflammation is not balanced optimally, the granuloma will necrotise centrally and become caseous [24], allowing the growth of very high numbers of *M. tuberculosis* bacilli. These liquefying granulomas can break and discharge their contents into the bronchioli, at which stage contagious TB has developed (fig. 1). Immunity to *M. tuberculosis* is thus a double-edged sword: it not only protects against disseminating infection, but is also involved in the immunopathology underlying active TB disease and transmission.

CD4+ T-cells

Which immune responses need to be induced by new TB vaccines? How is a balanced response achieved that promotes protection and avoids pathology? *M. tuberculosis* infection involves a myriad of different cell types, including macrophages, DCs, epithelial cells, classical and nonclassical T-cells, neutrophils, B-cells and natural killer (NK) cells. The role of CD4+ T-helper (Th) type 1 cells in TB is generally considered to be essential. Th1 cells secrete IFN-γ and tumour necrosis factor (TNF), which activate macrophages to inhibit and eliminate intracellular pathogens by inducing: phagosome maturation and phagosome–lysosome fusion; autophagy; apoptosis; MHC antigen processing and presentation; antimicrobial peptide activity; and the formation of reactive oxygen and nitrogen intermediates. Th1 cells are induced primarily by antigen presented in the context of IL-12 from phagocytes [9]. Illustrating this, genetic deficiencies in proteins essential to the induction (IL-12 p40 subunit and IL-12 receptor β1) or function (IFN-γ receptors

1 and 2, and STAT1) of Th1 cells, or the reduction of CD4+ Th1 cells due to HIV infection greatly enhance susceptibility to mycobacteria, including *M. tuberculosis* [9, 25]. Treatment with TNF inhibitors similarly enhances TB reactivation rates greatly [26].

The recently discovered Th17 cells are a second type of CD4+ Th cells that is important in TB. Th17 cells produce IL-17A, IL-17F, IL-22 and TNF, are pro-inflammatory, mediate antimicrobial immunity against extracellular bacteria and fungi, and regulate mucosal immunity. Th17 differentiation is dependent on IL-1β or IL-6, transforming growth factor (TGF)-β and, in most conditions, on IL-23, a molecule closely related to IL-12 [27]. Th17 cells are thought, especially, to play a role in early-phase protection against *M. tuberculosis* infection, at least in mice [28, 29]. IL-17A is essential to forming mature pulmonary granulomas in BCG or *M. tuberculosis* infection models [30]. However, the role of IL-17 in chronic and latent infection is less clear, and may be pathological rather than predominantly protective [31]; hyperimmunisation of previously *M. tuberculosis*-infected mice by repeated BCG vaccinations led to increased immunopathology, with IL-17-dependent recruitment of neutrophils and tissue destruction instead of infection control [31, 32]. Also, human evidence underscores the potential role of granulocytes in TB disease [33–35]; genetic profiling of TB patients showed a gene expression pattern that was dominated by a type-1 IFN signalling signature associated with neutrophils. Thus, Th17 cells and their downstream effector mechanisms may provide biomarker signatures of pathogenesis during *M. tuberculosis* infection.

"Multifunctional" CD4+ T-cells have been reported recently, multi- or polyfunctionality referring to the simultaneous production of multiple cytokines (mostly IFN-γ, IL-2 and TNF) or the expression of multiple effector functions (perforin, granulysin, cytolysis, *etc.*) by single T-cells. There is some discussion over whether polyfunctional CD4+ T-cells are associated with protection against TB or not. Several animal vaccination studies have suggested that polyfunctional (IFN-γ+ IL-2+ TNF+) CD4+ T-cells are associated with protective immunity [36, 37], whereas other, mostly human, studies have suggested that they may not clearly associate with protection [38–40]. Even though this issue needs further investigation, this seeming paradox could be explained by the fact that active TB is a heterogeneous disease, ranging from the reactivation of only a few lesions up to full-blown activation of many lesions, such as in rapidly disseminating disease. In mild and less severe active TB disease, some lesions will be active, while others may remain under adequate immune control, which may involve multifunctional CD4+ T-cells. Thus, multifunctional T-cells are not only induced by vaccination and associated with protection following vaccination, they may also be present during active TB disease as part of the protective host response that attempts, but does not succeed, to limit infection.

CD8+ T-cells

CD8+ T-cells also contribute to optimal immunity and protection against TB [41, 42]. The mechanisms underlying CD8+ T-cell activation in TB are incompletely defined [43]. Besides classical MHC class Ia-restricted CD8+ T-cells, MHC class Ib-restricted CD8+ T-cells also participate in the immune response to *M. tuberculosis* [44, 45]. These include: human leukocyte antigen (HLA)-E-restricted cells [46, 47]; lung mucosal-associated invariant T-cells, which recognise *M. tuberculosis* antigens in the context of the nonclassical molecule MR1 (MHC class I-related protein) [48]; and CD1-restricted T-cells, which mostly recognise lipid antigens derived from *M. tuberculosis*. HLA-E molecules are enriched in the *M. tuberculosis* phagosome, thus providing a plausible mechanism for *M. tuberculosis* peptide loading [48]. CD8+ T-cells possess multiple effector mechanisms to inhibit *M. tuberculosis* infection. Importantly, since all nucleated cells express MHC class Ia molecules, CD8+ T-cells should be able to recognise all cell types infected by *M. tuberculosis*, whereas CD4+ T-cells can only recognise MHC class II-expressing infected cells, which are typically macrophages and DCs. Moreover, since CD8+ T-cells recognise peptides originating from the cytoplasm of infected cells, they survey a compartment complementing that scanned by CD4+ T-cells; the latter primarily recognise antigens from the extracellular milieu, including antigens sequestered in the vacuolar system, such as phagosomes and phagolysosomes. CD8+ T-cells secrete cytotoxic molecules, such as perforin, granzymes and granulysin, which kill infected host cells and, in the case of granulysin, can kill *M. tuberculosis* and

other bacteria. CD8+ T-cells also induce apoptosis of infected target cells through Fas and other TNF receptor family-related cell death receptors. Human CD8+ T-cells can also produce the Th1 cytokines IFN-γ and TNF [49–55].

Regulatory T-cells

M. tuberculosis not only triggers CD4+ and CD8+ T-cells with antimicrobial effector functions but also regulatory T-cell (Treg) populations. These include naturally occurring CD4+ CD25+ FoxP3+ Tregs as well as *M. tuberculosis* antigen-specific induced CD4+ Tregs [56, 57] and CD8+ Tregs, mostly identified in humans [46, 58, 59]. Tregs have multiple inhibitory effects on CD4+ Th cell activity, including through IL-10, CCL4, membrane-bound TGF-β and deactivation of antigen-presenting cells These mechanisms preclude optimal priming of CD4+ T-cell responses, and can probably inhibit the recruitment of naïve CD4+ T-cells to the draining lymph nodes [56], thus delaying and inhibiting the onset of adaptive immunity to *M. tuberculosis*.

Avenues towards designing better TB vaccines

More than a dozen TB vaccine candidates have entered clinical testing, while several other promising high-profile candidates are in pre-clinical phase testing, and are being considered for clinical testing [3, 43]. There are five vaccines that are being tested in phase I/IIa (safety/immunogenicity) trials in humans. Moreover, six vaccines have been moved forward for testing in phase II trials, two of them already in phase IIb. Many more promising candidates have advanced to pre-clinical evaluation, and several more are in the TB vaccine research discovery pipeline.

There are two broad and non-mutually exclusive strategies for developing new TB vaccines [1, 60, 61]. The profile of such vaccines is that they should be safer, more immunogenic and induce longer-lasting protection than BCG, and preferably also induce protection against highly virulent clinical isolates, such as *M. tuberculosis* Beijing strains, as well as MDR, XDR and TDR *M. tuberculosis* strains. A first strategy to develop better TB vaccines is through subunit vaccines. These are non-live (or, in the case of viral vectors, at least nonreplicating) vaccines. A major advantage is that such vaccines should be safe for humans, including immunocompromised individuals. TB subunit vaccines consist of recombinant *M. tuberculosis* antigens combined with an adjuvant or of virally vectored *M. tuberculosis* antigens. Subunit vaccines are generally viewed as booster vaccines that can be given following previous priming with BCG (or recombinant BCG (rBCG)/attenuated *M. tuberculosis*, as will be discussed later). The aim of such subunit vaccines is to boost long-term memory responses in previously primed individuals. This is needed since BCG-induced immunity seems to wane over time, particularly during adolescence and in young adults, when risk of TB acquisition is highest.

The second strategy to develop better TB vaccines is to replace BCG with rBCG vaccines or genetically attenuated live *M. tuberculosis* vaccines. Such vaccines would replace rather than boost BCG. This also implies that such vaccines need to be given prior to *M. tuberculosis* infection, and thus should be administered to neonates and infants. Such vaccines should be safe and not interfere with the EPI. To improve BCG, several researchers have introduced *M. tuberculosis*-specific antigens into BCG that are absent from BCG, such as the region of differentiation (RD)1 locus, which encodes antigens 6-kDa early secretory antigenic target (ESAT-6) and 10-kDa culture filtrate protein. Others have overexpressed antigens that BCG already expresses, such as Ag85B. An alternative way to improve BCG is by introducing genetic modifications to allow rBCG to induce better immunity. This could be combined with genetic expression of protective antigens [23].

A challenging strategy to develop better live TB vaccines is the use of attenuated *M. tuberculosis* strains. This requires the deletion of essential metabolic genes to create auxotrophic mutants, or the deletion of virulence genes or their regulators (see later), preferably at least two independent genetic loci to avoid possible complementation and restoration of virulence. Others expressed *M. tuberculosis* RD1 antigens in *Mycobacterium microti* and were able to achieve improved protection in animal models [62]. Surprisingly, recombinant live mycobacterial vaccines with high activity

against *M. tuberculosis* can also be obtained using *Mycobacterium smegmatis* as a vehicle. The removal of the endogenous *esx-3* locus from *M. smegmatis* resulted in improved innate immune responses; when this *M. smegmatis esx-3* locus-deficient strain was further modified by the genetic transfer of the *M. tuberculosis esx-3* locus, a strong protective effect against *M. tuberculosis* was observed in vaccinated mice, in some cases even sterilising immunity [63].

TB subunit vaccines

As mentioned earlier, TB subunit vaccines are either virally vectored or recombinant antigens mixed with adjuvants. There are two virally delivered TB subunit vaccines in phase IIb clinical trials in Africa. The first, a replication-deficient modified Vaccinia virus Ankara vaccine expressing Ag85A, is highly immunogenic in humans (both in mycobacterially naïve as well as BCG-primed individuals) and is capable of inducing high Ag85A-specific CD4+ T-cell responses in humans [64, 65]. The second is a replication-deficient adenovirus 35-based delivery system, engineered to express the *M. tuberculosis* antigens Ag85A, Ag85B and TB10.4. This was also shown to be safe and highly immunogenic in humans, and induced strong CD4+ and CD8+ T-cell and IFN-γ responses [66, 67].

Major progress has also been made in the testing of recombinant TB protein vaccines. Thus far, these have been mostly fusion proteins combining two or more immunodominant antigens of *M. tuberculosis*, mixed with potent Th1-activating adjuvants. One candidate vaccine is the Hybrid 1 fusion protein from Statens Serum Institut (Copenhagen, Denmark). This vaccine consists of Ag85B fused to ESAT-6, which, when administered with the adjuvant IC31® (Intercell AG, Vienna, Austria) (which activates human Toll-like receptor (TLR)9 and facilitates antigen uptake by DCs [61]), induced strong CD4+ Th1 IFN-γ induction in humans (both in mycobacterially naïve as well as in BCG-primed or LTBI individuals) [68, 69]. The vaccine induced long-lasting CD4+ Th1 immunity, which lasted over 2.5 years after the last vaccination. This vaccine is now in phase IIa testing. The related fusion protein HyVac4, which consists of Ag85B fused to *M. tuberculosis* antigen TB10.4 (which, in contrast to ESAT-6, is also expressed by BCG), has a highly similar profile, and is in phase I clinical testing (table 1).

In a parallel effort, GlaxoSmithKline (London, UK) designed the M72 fusion protein, consisting of *M. tuberculosis* antigens Rv1196 and Rv0125 [70]. The M72 fusion protein, admixed with the synthetic adjuvant AS01, was found to be safe and immunogenic in humans [70]. AS01 contains monophosphoryl lipid A, which stimulates TLR4 and drives CD4+ Th1/Th17 responses, and QS21, which induces CD8+ T-cell responses. This vaccine is now in phase IIa clinical testing.

Another promising adjuvant currently being evaluated in phase I clinical trials is cationic adjuvant formulation (CAF)01 [71, 72]. This liposome-based formulation consists of dimethyl-dioctadecyl-ammonium combined with trehalose 6,6′-dibehenate, which activates the innate immune receptor monocyte-inducible C-type lectin [73].

The heparin-binding haemagglutinin (HBHA) represents another *M.* tuberculosis antigen, which is close to being tested in phase I clinical trials [74]. The *M. tuberculosis* HBHA antigen is methylated in its C-terminal portion, against which T-cell responses are mostly directed [75]. In addition, DNA-vectored vaccines are being considered, not only as booster vaccines but also, possibly, as therapeutic vaccines (table 2).

Besides the antigens mentioned here, many other antigens have been discovered that could be interesting vaccine candidates. Examples of these are *M. tuberculosis* latency antigens, such as DosR regulon-encoded proteins and starvation antigens such as Rv2660, which will be discussed in the section on post-infection/therapeutic vaccines.

Live vaccines aiming to replace BCG as priming vaccines

TULLIUS *et al.* [77] were the first to create rBCG strains for vaccination in humans. They overexpressed Ag85B in BCG, which was termed rBCG30 [78]. This vaccine was immunogenic in

Table 1. The most advanced tuberculosis (TB) vaccine candidates in clinical trials

Type of TB vaccine	Vaccine components	Vaccine description	Clinical trial status
Fusion protein in adjuvant for pre-exposure vaccination as booster on top of BCG	Hybrid 1 and IC31®	Fusion protein consisting of Ag85B and ESAT-6 administered in adjuvant IC31®	Phase IIa
	Hybrid 56 and IC31®	Fusion protein consisting of Ag85B, ESAT-6 and Rv2660c administered in adjuvant IC31®	Phase I ongoing
	Hybrid 1 and CAF01	Fusion protein consisting of Ag85B and ESAT-6 administered in adjuvant CAF01	Phase I ongoing
	M72 and AS01 or AS02	Fusion protein consisting of Rv1196 and Rv0125 administered in adjuvant AS01 or AS02	Phase IIa ongoing
	Aeras-404 (HyVac4 and IC31®)	Fusion protein consisting of Ag85B and TB10.4 administered in adjuvant IC31®	Phase I
Viral vector for pre-exposure vaccination as booster on top of BCG	Oxford MVA85A/ Aeras-485	MVA expressing Ag85A	Phase IIb ongoing
	Crucell Ad35/ Aeras-402	Replication-deficient Ad35 expressing Ag85A, Ag85B and TB10.4	Phase IIb ongoing
	AdAg85A	Replication-deficient Ad5 expressing Ag85A	Phase I
rBCG for pre-exposure vaccination as priming vaccine, replacing BCG	VPM1002	rBCG expressing listeriolysin and urease deletion	Phase IIa ongoing
	rBCG30	rBCG expressing Ag85B	Phase I completed/ on hold
	Aeras-422	rBCG expressing perfringolysin, Ag85A, Ag85B and Rv3407	Phase I terminated due to side-effects
Whole bacterial vaccines for therapeutic vaccination	RUTI®	Detoxified *M. tuberculosis* in liposomes	Phase IIa ongoing
	M. vaccae	Inactivated *M. vaccae*	Phase III completed

IC31® is manufactured by Intercell AG (Vienna, Austria). RUTI® is manufactured by Archivel (Badalona, Spain). BCG: bacille Calmette–Guérin; rBCG: recombinant BCG; CAF: cationic adjuvant formulation; MVA: modified Vaccinia virus Ankara; Ad: adenovirus; Ag: antigen; VPM: Vakzine Projekt Management (Berlin, Germany); *M. vaccae*: *Mycobacterium vaccae*; ESAT-6: 6-kDa early secretory antigen target; *M. tuberculosis*: *Mycobacterium tuberculosis*.

humans [79] and passed phase I clinical safety testing but is currently on hold. Other researchers followed the same basic strategy, transferring selected RD1 *M. tuberculosis* antigens or larger portions of the RD1 locus into BCG [80]. The resulting rBCGs were more immunogenic but also more virulent in the severe combined immune deficiency (SCID) mouse safety model. Deleting the virulence determinants in RD1 may improve the safety of these vaccines. Other studies used a rBCG strain overexpressing Ag85A, Ag85B and TB10.4 [80]. However, while safe and immunogenic in mice, this vaccine did not show improved protection against TB compared with the wild-type BCG.

Another approach aimed to improve the efficacy of BCG by heterologous expression of the *Listeria monocytogenes*-derived molecule listeriolysin (encoded by the *hly* gene), while deleting the

Table 2. The most advanced tuberculosis (TB) vaccine candidates in pre-clinical development and production

Type of TB vaccine	Vaccine	Vaccine description
Purified protein	HBHA	Methylated purified protein from *M. bovis* BCG
DNA vectored	HG85A Hsp DNA vaccine	DNA vaccine encoding Ag85A Codon-optimised *M. leprae* Hsp
Attenuated *M. tuberculosis*	MTBVAC	*M. tuberculosis* attenuated by stable deletion of *M. tuberculosis phoP* and *fadD26* genes
		M. tuberculosis auxotrophic for lysine and pantothenate and attenuated by inactivation of the *secA2* gene

M. tuberculosis: *Mycobacterium tuberculosis*; HBHA: heparin-binding haemagglutinin; Hsp: heat-shock protein; *M. bovis*: *Mycobacterium bovis*; Ag: antigen; *M. leprae*: *Mycobacterium leprae*. Data from [76].

endogenous BCG *ureC* gene [81]. Hly mediates phagosomal escape of *Listeria* to the cytoplasm by perforating the phagosomal membrane, concomitant with the release of host proteolytic enzymes and bacterial products into the cytosol. However, listeriolysin is only active at lower pH. Because UreC counteracts phagosomal acidification, the deletion of the *ureC* gene from BCG allows activity of Hly in the acidic milieu of the phagosome. rBCG lacking *ureC*, but expressing Hly, induced superior protection compared with parental BCG [81]. It was found to induce not only Th1 but also Th17 cells, which probably facilitate optimal immunity against *M. tuberculosis* [82–84], including virulent *M. tuberculosis* isolates. The rBCGΔ*ureC*::*hly* vaccine, now termed VPM1002 (Vakzine Projekt Management, Berlin, Germany), has successfully entered a phase IIa trial in newborns (table 1).

The genetic expression of perfringolysin in BCG was originally considered to have similar effects to Hly [80]. An important difference, however, is that perfringolysin has a broader pH optimum and remains active at neutral pH in the cytosol [23], and can act both inside and outside the cell. Moreover, Hly, but not perfringolysin, is degraded upon appearance in the cytosol [85]. Thus, Hly possesses inherent safety mechanisms absent from perfringolysin. A recent phase I clinical safety trial with rBCG expressing perfringolysin and Ag85A, Ag85B and Rv3407 (termed Aeras-422; Aeras, Rockville, MD, USA) [80, 86] was discontinued due to adverse effects (in particular, the reactivation of shingles [87]) (table 1).

An alternative approach to developing live vaccines has been through genetic attenuation of *M. tuberculosis*, either by deletion of essential metabolic genes to create auxotrophic mutants, or by deletion of (at least two) virulence genes (table 2). Auxotrophy can be achieved by deleting genes that regulate the synthesis of essential nutrients, such as amino acids or pantothenate [88]. Promising candidate vaccine strains for human testing have been constructed, such as a *M. tuberculosis* ΔRD1 Δ*panCD* strain, from which the RD1 virulence gene cluster was deleted next to the gene responsible for synthesising pantothenate [89]. This strain was protective in normal and immunocompromised mice, and appeared to be safe. The Δ*phoP* Δ*fad M. tuberculosis* strain [90] contains deletions in the two-component PhoP/PhoR transcriptional regulator system, which controls the expression of many *M. tuberculosis* genes, including virulence genes. In addition, the *fad* gene regulates the synthesis of the essential *M. tuberculosis* cell wall lipid phthiocerol dimycocerosate. The Δ*phoP* Δ*fad M. tuberculosis* vaccine candidate will enter phase I clinical safety testing in 2012.

Towards better TB vaccines: what should next-generation vaccines look like?

Post-infection or therapeutic vaccines

New TB vaccines should not only induce immune responses to control newly acquired infection but, ideally, also eradicate *M. tuberculosis* organisms from the human body or at least achieve permanent

control of latent infection to prevent TB reactivation [60]. Most TB vaccines are pre-exposure candidates based on strong, long-lasting memory, and aim to induce strong, long-lasting memory T-cell responses against early phase-expressed *M. tuberculosis* antigens. However, it has become clear that *M. tuberculosis* switches from active replication to slow or nonreplication and enters into a metabolic shift-down during infection, accompanied by large changes in gene expression. The expression of the ''early secreted antigens'' by replicating *M. tuberculosis* is decreased whereas the expression of so-called latency antigens is induced or increased. *M. tuberculosis* latency antigens can be encoded by the DosR regulon, which controls the response to hypoxia, or by starvation or nutrient stress antigens, which are upregulated upon nutrient deficiency [91–96]. New studies have used such starvation/latency antigens to improve live as well as subunit vaccines. Vaccination with a fusion protein of Ag85B, ESAT6 and the starvation/latency antigen Rv2660c induced superior long-term protection against TB both in mice [97] and in a nonhuman primate model [98]. This vaccine, termed H56, has entered phase I clinical safety testing (table 1). Expression of latency antigens in rBCG also improved protective efficacy in a long-term TB mouse infection model [99].

Alternative possibilities that could be considered include the incorporation of other *M. tuberculosis* antigens, such as those that are expressed during reactivation. An example of such antigens is represented by resuscitation-promoting factor antigens and further downstream antigens [54, 86, 93]. The triggering of T-cell responses and immunological memory against such antigens may help to mount an early adequate host response to better control reactivating *M. tuberculosis*.

TB biomarkers: important tools to evaluate TB vaccines

The availability of biomarkers of TB vaccine efficacy will be of key importance to improve and accelerate TB vaccine development. At this stage, however, there are only biomarkers of TB vaccine immunogenicity available, not efficacy. In the absence of reliable TB biomarkers of protection and a human challenge model, TB vaccine design, by necessity, will have to rely on empirical testing of candidate vaccines in long and costly efficacy trials [1, 100]. The development of robust and reliable *M. tuberculosis in vitro* inhibition assays, as well as the first steps that are currently being taken towards a human *in vivo* challenge model using BCG, are important in measuring outcomes relevant to infection and vaccination, rather than immunogenicity alone. The identification of protective biomarker signatures would greatly facilitate vaccine discovery and testing [101, 102]. TB vaccine research should, therefore, also invest in TB-biomarker discovery and testing. As long as we do not know the exact mechanisms of protective immunity in TB and do not have biomarkers available, it may be best to develop vaccines that stimulate a wide variety of potential effector mechanisms, including Th1 cells, Th17 cells, CD8+ T-cells, perhaps T-cell receptor γδ cells, NK T-cells [103], and perhaps also *M. tuberculosis* lipid-reactive T-cells, all of which can express multiple effector functions against *M. tuberculosis*, which could complement each other.

Closing remarks

There are a number of high-profile TB vaccine candidates that have already entered clinical trials, with promising immunogenicity profiles. However, in the absence of surrogate end-point biomarkers of protection, we cannot predict whether these vaccines will have protective efficacy in humans, regardless of their excellent records in animal models [1, 104]. Thus, phase III efficacy trials will be needed to test the vaccines and determine their efficacy. Other vaccine candidates are likely to enter clinical testing soon.

Future vaccines should be considered that can prevent infection or induce sterile eradication, such as by redirecting immune responses towards *M. tuberculosis* antigens expressed during latency [91–93, 97] or by alternative means [64]. Thus, we are currently witnessing promising developments in the field of TB vaccines: interesting TB vaccine candidates in clinical testing, and more promising candidates in the development pipeline.

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Chapter 6

Prevention of TB in areas of low incidence

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SUMMARY: Identifying particular groups that are at high risk of developing tuberculosis (TB) is of major interest in low-incidence countries. In order to reach the phase of TB elimination, new challenges arising from the steadily declining TB prevalence in the native population but increasing importation of *Mycobacterium tuberculosis* strains from high-incidence countries and the growing emergence of hard-to-reach groups, must first be dealt with. Thus, in particular, the evidence of protection by bacille Calmette–Guérin (BCG) vaccination and screening of migrants has to be assessed. A newly recommended regime for preventive treatment of latent TB infection (LTBI) in HIV- and non-HIV-infected persons is promising. However, a rapid evaluation of possible strategies of preventive treatment for contacts of multidrug-resistant (MDR) source cases is still lacking.

Prevention of TB in low-incidence countries is complex and no single action can lead to a lasting result but rather a bundle of properly balanced measures must be taken.

KEYWORDS: Bacille Calmette–Guérin vaccination, immigrant screening, latent tuberculosis, multidrug-resistant tuberculosis, targeted testing

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The epidemiological situation in respect to tuberculosis (TB) in most Western countries has shown favourable development over the past several decades through a 10–100-fold decline in the rates of TB thanks to better social conditions and improvements in case detection and treatment. As a result, TB prevention measures are no longer designed to cover the entire population, but rather to enable the early detection of index cases in high-risk groups and to reduce the prevalence of latent TB infection (LTBI) in persons with an increased risk of progression to active disease. This is all the more important as recent molecular epidemiology has demonstrated that, even in countries with a low incidence of TB, 30–40% of all cases can be expected to be newly transmitted and 13–16% of TB recurrences were the result of exogenous re-infection [1].

Most of the groups for which intervention may be highly effective have been clearly defined for decades now and surveillance in low-incidence industrialised countries is well established by National Tuberculosis Control Programmes (NTPs) at both the national and subnational level, reporting similar indicators [2], but the preferred methods of TB prevention and control applied to such risk groups are often a matter of controversy.

Meanwhile, the incidence of TB is low in most industrialised countries, defined by the World Health Organization (WHO) as a crude notification of less than 20 cases per 100,000 population. To further achieve the goal of TB elimination (when incidence of sputum smear-positive TB cases becomes less than one case per 100,0000 population), some key operations have to be performed in a balanced combination.

In this chapter, we discuss the most important elements of TB prevention and control in low-burden countries and recommend some issues for further research.

Targeted testing and treatment for LTBI

Targeted testing and treatment for LTBI are important cornerstones of the TB control strategy to prevent and control TB in low-incidence countries [3]. Of all the American Thoracic Society (ATS)/Centers for Disease Control and Prevention (CDC)-defined groups at high risk of developing TB, contact persons with a presumed recent *Mycobacterium tuberculosis* infection are most likely to benefit from treatment for LTBI. For all persons, the first 2 years following infection are the most vulnerable with regards to possible progression to disease, but among those contacts, children and HIV-infected individuals are most vulnerable and require special attention. Considering the generally weaker immune system of children, without preventive treatment, disease progression is often faster than is the case in adults, and up to 40% of infants develop the disease once infected [4]. For the USA, the lifetime risk of progression has been calculated to be 10–20% among children aged 5 years or younger who have an induration of $\geqslant$10 mm on a tuberculin skin test (TST) [5].

Young adults (aged 15–49 years) with recently diagnosed TB are nearly 19 times more likely to be co-infected with HIV than those without TB and, conversely, people living with HIV are 20–30 times more likely to develop TB than those without HIV [6].

Preventive treatment has been shown to prevent TB in individuals in recent close contact with persons infected with *M. tuberculosis*, children, HIV-positive individuals with LTBI, and diverse categories of patients who are otherwise at a relatively higher risk of infection and/or disease compared with the healthy population. These categories comprise other immunocompromised individuals, *e.g.* people who are undergoing immunosuppressive treatment, patients with chronic kidney failure or silicosis, but also the homeless, intravenous drug users and immigrants that may contribute in higher numbers to the pool of latent infections that has to be reduced [3].

TB incidence is higher among persons older than 50 years compared with younger persons (risk ratio (RR) 3.8, 95% CI 1.3–11) [7], but here, lifetime risk of reactivation in the case of a recently converted TST is only 2–4% [5], thus not justifying this as a priority for LTBI treatment.

With respect to the regimens available for treating LTBI, protective efficacy may achieve 90% ranges depending on the duration and adherence to the most extensively examined isoniazid therapy [8], but effectiveness in routine practice has been shown to be clearly limited. Self-supervised daily isoniazid regimes have completion rates of 60% or less in typical settings, attributable largely to poor adherence to the required duration of at least 6 months. Adherence may be reduced further as in most low-incidence countries it is recommended that even isoniazid should be given daily for 9 months in high-risk individuals with evidence of LTBI. Rare but severe liver injuries and, probably, the concerns over this risk have reduced acceptance of these regimes, which require some monitoring, and so the disputes over balancing the risks and benefits of preventive therapy still remain.

To date, there are only two meta-analyses available that address the rate of isoniazid-induced hepatitis in adults. The first one, by Steele *et al.* [9], reviewed six very different studies on the incidence of hepatitis in adults taking isoniazid therapy (including the Capitol Hill (Washington, DC, USA) investigation [10], the United States Public Health Service (USPHS) surveillance study [11] and the Eastern European International Union Against Tuberculosis (IUAT) study [12]) published between 1965 and 1984. In its pooled data they provided a summary rate of clinical hepatitis of 0.6% (210 out of 38,257 patients). The meta-analysis by Smieja *et al.* [8], using only

randomised studies and fulfilling the evidence-based criteria of a meta-analysis, only selected the Eastern Europe IUAT study which is, to date, the biggest prospective single study determining the incidence of isoniazid hepatitis. In this study, patients had a rate of isoniazid-associated hepatotoxicity of 0.36% (6 months treatment) and 0.44% (9 months treatment) based on related clinical symptoms. Also the early USPHS study performed between 1971 and 1973 documented 82 probable cases of isoniazid hepatitis among 13,838 patients receiving isoniazid (0.6%) [11].

In fact, newer but smaller studies including treatment of HIV-infected persons have reported a grade 3 or 4 hepatitis in up to 5.3% (table 1 [13–26]) in those treated with isoniazid, and the recently published randomised study by STERLING *et al.* [27] presents a comparatively high proportion of hepatitis in 2.7% of the 3,745 isoniazid recipients.

Shorter course regimes than the current 9-month isoniazid regime for persons with newly diagnosed LTBI and those that use directly observed therapy (DOT) in order to maximise adherence are key to reducing default from treatment and enhancing the success of preventive treatment. Two large recently published randomised controlled trials [27, 28] have impressively demonstrated that a new combination regime of isoniazid and rifapentine administered once weekly for 12 weeks as DOT *versus* self-administered isoniazid given daily for 9 months is less toxic and more likely to be completed. This new regime also prevents TB in at least an equivalent percentage as the 9 month isoniazid course (92% compared with 88%, respectively) and was recently recommended in the USA [29]. Although rifapentine, a rifamycin with a prolonged half-life that is also available for the European market too, is associated with higher costs at first glance, this new combination can be considered a more effective and less expensive alternative for high-risk individuals under DOT, particularly those with HIV, and will be cost-effective even for lower risk patients [30].

As far as the detection of LTBI before offering treatment is concerned, until recently the TST, which may be confounded by bacille Calmette–Guérin (BCG) and non-tuberculous mycobacteria (NTM) and accordingly can produce false-positive reactions, was the primary method used to diagnose LTBI. The introduction of the new highly specific interferon-γ release assays (IGRAs) was met with great enthusiasm as it could help determine the number of people who could actually benefit from preventive therapy. Because IGRA screening requires only one visit to obtain and interpret results, IGRAs may also minimise losses to follow-up and increase receipt of test results [31]. However, guidelines vary as to whether the more expensive IGRAs should be used as a primary or confirmatory test for identifying LTBI [32].

There is some evidence to suggest that commercial IGRAs are more reliable than the TST for predicting TB among high-risk persons. In a systematic review in which conclusions were drawn from just seven studies (three of which directly compared "in house" IGRAs and the TST) the incidence RR was significant for the IGRAs but not for the TST, showing a more than doubled risk for developing active TB given a positive IGRA. However, the overall ability of IGRAs or TST to predict active TB statistically was "much the same" [33]. In another recently published meta-analysis the pooled positive predictive value (PPV) for progression for the 17 included studies using commercial IGRAs was clearly higher, but overall still only modest, with a PPV of 6.8% for the IGRAs compared with 2.4% for the TST [34].

Therefore, these analyses reveal the major limitations of current LTBI screening and prevention strategies, namely that many high-risk persons must still be treated to prevent a single TB case as none of the currently available tests for LTBI, beyond the respective test-related characteristics of more or less higher specificity, can accurately predict who will develop active TB. Thus, the search for better LTBI tools which have the capacity to predict who of those truly infected will progress to active TB has to be continued.

Immigrants from countries with a high incidence of TB

In the USA and Western Europe, more than 50% of those persons who suffer from TB are foreign-born, mainly younger persons, whereas in natives, TB disease mainly affects the older age groups [35, 36].

Table 1. Severe hepatotoxicity (clinical hepatitis or grade 3–4 hepatotoxicity) in studies of isoniazid (INH) treatment of latent tuberculosis (TB) infection

First author [ref.]	Years	Setting	Type	Regimen	Subjects evaluated n	Cases n	Hepatotoxicity %
GARIBALDI [10]	1970	Capitol Hill, Washington, DC, USA	Observational cohort of employees in TB outbreak	INH 300 mg 6 months	2321	19	0.8
KOPANOFF [11]	1971–1972	Public health departments, 21 cities, USA	Observational cohort	INH 300 mg 6 months	13838	82	0.6
THOMPSON [12]	1969	115 clinics in 7 Eastern European countries	Controlled, double-blind trial	INH 300 mg 3–9 months	20840	95	0.46[#]
NOLAN [13]	1989–1995	Seattle King County Public Health Department, WA, USA	Observational cohort	INH 300 mg 6 months	11141	11	0.1
JASMER [14]	1993–1994	San Francisco, CA, USA TB clinic	Prospective, open-label trial	INH 300 mg 12 months	545	6	1.1
JASMER [15]	2000	3 urban public health TB clinics, USA	Multicentre, prospective, open-label trial	INH 300 mg 6 months	204	2	1.0
MCNEILL [16]	1999–2001	Pitt County Health Department, NC, USA	Observational cohort	INH 300 mg 6 months	114	5[¶]	4.4
LOBUE [17]	1999–2002	San Diego TB Clinic, CA, USA	Observational cohort	INH 300 mg 6–9 months	3788	10	0.3
LEUNG [18]	2000–2002	Pneumoconiosis clinic, Hong Kong	Open-label, randomised trial	INH 300 mg 6 months	36 in INH arm	1	2.8
MENZIES [19]	2002	Respiratory hospital, Montreal, Canada	Open-label randomised controlled trial	INH 300 mg 9 months	58 (INH arm)	3	5.2
FOUNTAIN [20]	1996–2003	Public health clinic, Memphis, TN, USA	Observational cohort (patients aged ⩾25 years)	INH 300 mg 6 months	3337	19	0.6
TORTAJADA [21]	2001–2003	9 public health care centres from Barcelona, Madrid, La Coruña and León, Spain	Multicentre, randomised trial (any patient aged <35 years)	INH 5 $mg \cdot kg^{-1} \cdot day^{-1}$ (maximum 300 mg) 6 months	159 (INH arm)	5	3.1
SPYRIDIS [22]	1995–2005	Paediatric TB clinic in Athens, Greece	Randomised controlled trial (children aged <15 years)	INH 5 $mg \cdot kg^{-1} \cdot day^{-1}$ (maximum 300 mg) 9 months	232 (INH arm)	0	
MENZIES [23]	2004–2007	TB clinics in Brazil, Canada and Saudi Arabia	Multicentre, randomised, open-label trial	INH 300 mg 9 months	422 (INH arm)	17	4.0
HAWKEN [24]	1992–1994	3 study clinics in Nairobi, Kenya	Randomised clinical trial in HIV-infected persons	INH 300 mg 6 months	342 (INH arm)	18[+]	5.3
WHALEN [25]	1993–1995	5 medical clinics and counselling centres, Kampala, Uganda	Randomised, placebo-controlled trial in HIV-infected persons (aged ⩾18 years)	INH 300 mg 6 months	536 PPD positives plus 395 anergic persons	1 PPD positive plus 6 PPD negative	0.8 (7 out of 931)
GORDIN [26]	1991–1996	Outpatient clinics in the USA, Mexico, Haiti and Brazil	Open-label controlled trial (HIV-infected, aged >13 years)	INH 300 mg 12 months	792	26	3.3

PPD: purified protein derivative. [#]: 0.25% after 3 months, 0.36% after 6 months and 0.44% after 9 months of treatment. [¶]: hepatotoxicity defined as alanine aminotransferase >160 $U \cdot L^{-1}$. [+]: no grade 3 or 4 hepatotoxicity. Biochemical hepatitis defined as aspartate aminotransferase greater than twice the upper limit of normal; no subject developed clinical hepatitis.

Currently, the rate of TB among the foreign-born population in the UK is as much as 21 times higher than the rate for the UK-born, focusing, in particular, on cities such as London, where a doubling of TB cases in the past decade has been attributed to immigration from high-prevalence countries [37]. Furthermore, the high rates of disease in those born in the UK whose parents or grandparents came from high-incidence countries have partly been responsible for the absence of a lasting decline in TB incidence in the UK-born population [38].

Thus, identifying and treating active TB in immigrants to low-incidence countries appears to be an interesting option for TB control, especially among new entrants, who have only a few mandatory points of contact with the healthcare authorities.

In fact, if based on chest radiograph examination and investigation of sputum smears, the contribution of routinely screening new immigrants for active disease is limited as it usually identifies only very few persons with active TB for every thousand immigrants examined, and only a few of these individuals are infectious. In a Swiss post-migration follow-up, only 43 out of 42,601 persons screened upon entry presented active TB disease, the majority of them showed no clinical signs at all, and the yield of acid-fast staining (AFS) was low, so the introduction of PCR testing for *M. tuberculosis* was requested for this particular situation [39].

In the USA, regulations require all immigrants to undergo TB screening in their countries of origin, and entering the USA is only permitted if sputum smears are negative in individuals with pulmonary infiltrates. However, more than 80% of new immigrants to the USA who were subsequently diagnosed with active TB were screened within 6 months before their arrival in the USA and had negative results, and 50% were given a positive diagnosis within 30 days after arrival [40]. Accordingly, the ability of current overseas screening programmes to detect TB based on chest radiographs and AFS was considered to be low, and missed individuals with AFS-negative active TB, who constitute the majority (65.1%) of new immigrants to the USA [41].

In a systematic review of the literature regarding immigrant screening in the European Union (EU) and Switzerland [42], including 22 publications between 1982 and 2008, the pooled estimate for case findings was 3.5 cases per 1,000 (95% CI 2.9–4.1), and there was no significant difference between screening at the port of entry, just after arrival or community post-arrival screening. Of note, in other low-incidence countries post-arrival screening is not always performed, *e.g.* in Germany, asylum seekers and refugees must obtain a chest radiograph only if they are looking for shared accommodation [43].

As active screening programmes are currently being debated because of their costs and the limited evidence that they reduce the transmission of TB in the host country, this has led to calls for LTBI screening and the provision of preventive treatment for immigrants in recent years in order to reduce the burden of TB [44]. Screening for LTBI in immigrants to low-burden countries is important because the majority of TB cases arise through the reactivation of infections acquired abroad. Although the still-valid US guidelines recommend screening and treatment solely for foreign-born persons living in the USA for 5 years or less [3], nearly half of the TB cases in the USA occur in foreign-born persons who have been in the USA for more than 5 years, and most of these cases are due to the reactivation of a latent infection [31].

LTBI screening has traditionally been carried out using the TST and the more recent IGRA tools might be a helpful tool in LTBI screening, as LTBI may be overdiagnosed if there is an over-reliance on the TST. However, as stated previously, the cost-effectiveness of programmes detecting and treating LTBI is also dependent on the extent to which treatment is completed. A recent cost-effectiveness analysis suggested that in the USA, screening immigrants for latent infection using IGRAs is more cost-effective than using the TST [31]. Also, under the assumption that completion rates varied between only 44% and 58% in different age groups in both recent immigrants and foreign-born residents who have lived in the USA for more than 5 years, IGRA screening resulted in either cost savings or in extended life expectancy with a lower cost per quality-adjusted life-year gained compared with TST, reflecting the impact of BCG vaccination on TST specificity and that IGRAs reduced the loss to follow-up.

In the UK, since 2006, the National Institute for Health and Clinical Excellence (NICE) [45, 46] guidelines for new-entrant TB screening currently recommend chest radiography for immigrants from countries with TB incidence >40 cases per 100,000 population. In the case of normal chest radiograph results, TST shall subsequently be performed followed by an IGRA for those with positive TST to confirm the diagnosis of LTBI.

In contrast, an IGRA-first protocol followed by chest radiography has been suggested to gain more LTBI cases and to be more cost-effective than the NICE guideline for screening new entrants from countries with TB incidence >150 cases per 100,000 [47] or >200 cases per 100,000 population [48].

Thus, whether this major potential benefit of immigrant screening in order to detect LTBI can be realised in practice needs to be confirmed in well-designed long-term studies while also taking into account the inevitable differences between countries and immigrant groups with respect to age, origin, national policies for the offer and incentives for completing preventive therapy.

BCG vaccination

BCG vaccination has been in use since its implementation in 1921 and has remained a part of the WHO Expanded Programme on Immunisation since 1974. BCG has been intensively used in high-burden countries where BCG vaccination at birth or within 1 year of birth is currently mandatory in 22 countries [49].

Unfortunately, the protective efficacy of BCG in terms of reducing disease prevalence varies considerably and the reason for this is not yet fully understood. Although vaccination with BCG has been shown to decrease the risk of severe forms of TB in young children by providing good protection (75–80%) against disseminated TB and meningitis [50, 51], it does not prevent infectious pulmonary TB, which occurs mainly in adults and remains the primary source of transmission. From a systematic review published in 1998, there is evidence that the protection after vaccination declines over time and may not last longer than 10 years [52].

As a result of the uncertain efficacy in adults and increasing concerns for adverse events following BCG vaccination, *e.g.* post-vaccination ulcers and lymphadenitis, very different vaccination policies have been implemented in industrialised countries in the last decades. The USA, Canada, Italy and the Netherlands have never implemented BCG vaccination on a national level and nine countries ceased universal BCG vaccination programmes between 1981 and 2007 [49]. In Germany, BCG vaccination has no longer been recommended since March 1998. Given the low incidence of TB among children and young people aged under 15 years in Germany on the whole (1.4 cases per 100,000 children, or 158 cases in 2010), tuberculous meningitis in particular (two cases in 2010), has now reached insignificant levels [53].

A model estimation by Manissero *et al.* [54] showed that a universal BCG programme could be beneficial in settings with a prevalence of at least 30 positive sputum smears per 100,000 inhabitants while in prevalence levels below five cases per 100,000 inhabitants the benefit of universal BCG vaccination may lead to an excess of adverse effects compared with the number of cases prevented.

With respect to a policy change towards selective high-risk group vaccination, the current Dutch strategy of targeting immigrant children from high-incidence countries and additionally including children from three lower-incidence, but higher-immigration countries, was shown to be cost-effective [55]. In France, however, where universal mandatory BCG vaccination was suspended in 2006 in favour of a targeted vaccination for some risk groups, the targeted vaccination policy did not gain acceptance, with less than half (44%) of the children eligible for BCG vaccination actually vaccinated [56].

Therefore, if national programmes are to consider BCG vaccination as part of a package of control measures in low-incidence countries for specific subgroups, not only for infants but also for immigrants from high-burden countries or for employees of the healthcare system working with drug-resistant (DR)-TB patients, a better vaccine is required.

There are currently 12 vaccines in development, or which have already gone into clinical trials, aimed at replacing the present BCG vaccine or at enhancing immunity induced by BCG. However, any new vaccine will be used in patients with LTBI, *i.e.* they are designed to prevent disease and will therefore neither eradicate the pathogen, nor prevent stable infection [57]. Therefore, the challenge in the near future will be to evaluate not only the safety and efficacy but also the correct timing of their administration in previously vaccinated persons, using long-term monitoring to confirm impact at the population level. About €560 million are considered to be necessary to achieve this result in the EU [58].

Preventive therapy in HIV-infected persons

In a recent study, HORSBURGH *et al.* [7] demonstrated that while the rate of TB reactivation from LTBI had decreased to no more than 0.040–0.058 cases per 100 person-years in HIV-negative individuals in the 1990s to 2000s, HIV-infected people were most at risk of reactivation, depending on the extent of their immunodeficiency.

In HIV-positive people with LTBI, HIV infection accelerates reactivation due to the failure of immune responses to restrict the growth of *M. tuberculosis*, so about 30% of people will eventually get active TB, also resulting in an increase in the risk of premature death [59].

Protective efficacy of 9-month isoniazid or multiple-drug regimes, given for 2–3 months (rifampin with isoniazid and/or pyrazinamide), is 60–80% in the short term [60], but the duration of protection may be shorter in HIV-infected persons than in non-HIV-infected individuals. Of note is a recently published meta-analysis where the effect of preventive therapy (any anti-TB drug) *versus* placebo was more pronounced and associated with a lower incidence of active TB (RR 0.68, 95% CI 0.54–0.85) in individuals with a positive TST (RR 0.38, 95% CI 0.25–0.57) than in those who had a negative test (RR 0.89, 95% CI 0.64–1.24) [59].

Antiretroviral therapy (ART) slows the development of immunodeficiency in HIV-infected persons and may delay the progression to active TB if a certain level of immunity can be restored. However, it is unclear whether this treatment reduces the lifetime risk of TB in such individuals.

According to current CDC recommendations, all HIV-positive individuals should receive a test for TB infection as soon as possible after HIV infection is diagnosed and at least every 12 months thereafter [61]. In the USA, in 2009, of those individuals with TB who had a documented HIV test result, more than 10% (690 of 6,743) were also co-infected with HIV; however, overall data on co-prevalence in the individual low-incidence countries is insufficient and may vary considerably (*e.g.* in Germany, it is estimated to be only 3%) [62].

However, the possible loss to follow-up of HIV-infected persons during the necessary steps from identification of those eligible to the completion of preventive therapy is not the only concern. Therapy for active TB cases among HIV-positive individuals, which was analysed by MIGLIORI *et al.* [63], is another point that must always be taken into account. Their findings documented that in an investigation of consecutive TB case samples in EU countries, TB/HIV co-infection was not satisfactorily managed in 34.8% of cases; in particular, ART was not prescribed in agreement with the existing recommendations, while in those cases that involved combined ART and anti-TB therapy there were no adverse effects.

In addition, a recently published systematic review [64] and trial [65] indicated that therapy should be generally prolonged to 9 months to reduce the proportion of relapses that are otherwise unacceptably high in HIV co-infected individuals.

Priority of multidrug-resistant TB in low-incidence countries

From an epidemiological point of view, primary infections with drug-susceptible TB strains result in partial immunity against exogenous re-infection with a different strain, and thus a population

may become more vulnerable to the spread of multidrug-resistant (MDR)-TB when the prevalence of LTBI has been reduced. Any low-incidence population that has successfully lowered its burden of drug-susceptible TB will have reduced herd immunity to externally imported or internally produced strains of MDR-TB and may subsequently experience heightened vulnerability to an epidemic [66].

Currently, incidence of MDR-TB in low-incidence countries is still low, ranging between 0 and 6.4% in low-incidence European countries [67], and in the USA the percentage of primary MDR-TB did not increase beyond 1.2% in 2010 [68]. The latter can also be contributed to the well-implemented DOT programme in US cities, which is known to reduce the frequency of acquired drug resistance [69, 70]. However, in the USA the proportion of reported primary MDR-TB cases occurring in foreign-born persons increased from 25.3% in 1993 to 82% in 2010 and in the EU, treatment outcome data showed that among all laboratory-confirmed MDR-TB cases in the 2007 cohort only 32% had a successful outcome at 24 months [71]. The risk of MDR-TB, defined as resistance to at least rifampin and isoniazid, correlates with insufficient treatment of a previous TB infection. In hard-to-reach risk groups, such as socially deprived patients with substance abuse problems and/or homelessness, drug-susceptibility testing (DST) for all patients and providing appropriate treatment is difficult due to limited accessibility and poor adherence and requires the special attention of public health authorities. Furthermore, with respect to the increasing burden of MDR-TB in high-prevalence countries, it is apparent that MDR-TB does not respect low-incidence country boundaries.

The key is not only managing the treatment of existing cases appropriately in order to contain the spread of this disease, which requires a high level of experience and a modern laboratory, but also the treatment of persons exposed to MDR-TB cases that have become latently infected with these strains; and above all this exposure has to be properly managed.

The evidence for successful drug combinations for the treatment of active disease is not in doubt, especially in low-incidence countries where resistance testing for targeted treatment regimens are regularly available, however; experience with the treatment of the respective contact persons is still insufficient at the moment. In line with an existing Cochrane review on that topic [72] showing that, to date, no randomised controlled trials have been published on the efficacy of possible treatment combinations for latent infection, the most recent European Centre for Disease Prevention and Control (ECDC) guidance [67] did not give a clear recommendation for preventive treatment either but demanded more evidence for recommending a concrete regime.

The alternative to preventive therapy is careful clinical follow-up of the identified contact considered to be latently infected with a MDR strain in order to speed up the detection of symptoms after the disease has eventually developed and to enable the initiation of a proper TB disease therapy as early as possible. However, that would require a reliable and stable relationship between the respective contact individuals and the public health authorities responsible for monitoring those patients and/or free and unbureaucratic access to clinical institutions. In low-incidence countries, the fact that vulnerable groups known to be at higher risk for TB, and subsequently the MDR cases, are increasingly concentrated in urban areas [38] poses a particular challenge. Thus, the maintenance of a well-established public health system in low-incidence countries is urgently needed despite the current economic crisis in which the national budgets for healthcare systems are being put under pressure.

TB risk and tumour necrosis factor-α inhibitors

At present, in line with remarkable advances in the therapeutic approach to patients with various chronic inflammatory and autoimmune diseases, a new population of patients is being threatened. Newer tumour necrosis factor (TNF)-α inhibitors (*e.g.* etanercept, infliximab and adalimumab) that greatly enhance the functional status and quality of life of those suffering from the treated diseases may also weaken the formation of protective granulomas at the site of mycobacterial infection.

TNF-α is a key pro-inflammatory cytokine and an essential component of host immunity required to maintain TB in the latent phase.

Since the introduction of TNF-α antagonists an increase in the cases of TB within the treated population has been observed. In the USA, TB rates have increased from 6.2 cases per 100,000 in the general population to 144 cases per 100,000 in patients who are receiving treatment with infliximab and 35 cases per 100,000 in those who receive etanercept [73]. Due to the high costs of TNF-α inhibitor treatment only patients from low-incidence countries can afford that therapy and accordingly are affected from possible TB reactivation without adequate screening for and treatment of LTBI [74].

Thus, as is the case before starting other immune system suppressing therapies, *e.g.* for patients awaiting transplants, precautionary measures must be taken before initiating TNF-α inhibitor therapy, *i.e.* patients should have active TB ruled out and screening for LTBI should be performed.

It has been demonstrated that strategies to treat patients with isoniazid 4 weeks prior to treatment with TNF-α inhibitors, usually given for a total of 9 months are effective in terms of decreasing the TB rate by 78% [75]. Nevertheless, besides mandatorily performing a chest radiograph examination and complete medical history, the recommendations for which test should be used to detect LTBI are highly heterogeneous. TST, which was commonly performed in the past, can be falsely positive and, hence, in four low-incidence countries (Germany, France, Austria and Switzerland), it is recommended only for exceptional situations and IGRAs are favoured. However, false-negative results in IGRA testing are also not uncommon and the other low-incidence countries recommend the use of either the TST or the IGRA, the use of both tests simultaneously, or alternatively a dual-step approach, *i.e.* IGRA if TST is positive (Spain and Norway) or IGRA if previous TST is negative (Canada and Italy) [32]. Consequently, further comparative longitudinal studies are needed to provide a more evidence-based estimate of the risk for progression to TB after IGRA- and/or TST-based diagnosis of LTBI in those patients starting therapy with TNF antagonists.

Molecular epidemiological analyses

The analysis of clusters of identical *M. tuberculosis* strains using DNA fingerprinting (*e.g.* restriction fragment length polymorphism (RFLP) method or spoligotyping) can provide retrospective information on the further spread of TB in local risk groups, particularly when the transmission of the disease cannot be satisfactorily determined using conventional surveys in difficult social circumstances (*e.g.* alcoholics and people who are in the country illegally) [1, 76]. This also allows the effectiveness of the selection of the ''correct'' contact people to be checked, by comparing the links, identified through fingerprinting, between the index case and any contacts who contracted the disease later. Thus, the combined use of epidemiological and strain typing data may be a powerful tool in the detection of recent transmission and, thus, the implementation of targeted public health control measures.

Unfortunately, at present, with the exception of Denmark, the Netherlands and the USA, in which cultured *M. tuberculosis* complex strains have been analysed from 1992, 1993 and 2004 onwards, in most low-incidence countries, these analyses are not performed as a matter of routine but rather within the framework of epidemiological studies [1]. Recently, in England and Wales (UK), a nationwide TB strain typing service has now been implemented for a course of at least 3 years.

Conclusions

Prevention of TB in low-incidence countries is dependent on efforts to tackle the disease in high-risk groups as well as to ensure that the health system is able to diagnose cases, regardless of group, as early as possible. The latter can only be achieved with high levels of awareness among physicians and an acceptance that TB is possible in any patient. It is critical that each country attempting TB

elimination reviews all elements of its control programme with a view to ensuring that a strategic approach is taken to address the locally relevant issues.

Statement of Interest

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Chapter 7

Prevention of TB in areas of high incidence

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SUMMARY: In areas where the tuberculosis (TB) epidemic is well controlled, most new cases are imported or result from endogenous re-activation of distant infection. In this scenario, the wide-scale use of preventive therapy could eliminate the pool of latent infection and facilitate TB eradication. However, in settings with poor epidemic control and ongoing *Mycobacterium tuberculosis* transmission, re-infection limits the ability to eradicate the pool of latent infection and reduces the duration of protection provided by preventive therapy. The provision of preventive therapy to vulnerable individuals following documented TB exposure and/or infection is universally accepted as the standard of care but multiple barriers result in a pronounced policy–practice gap, with near-absent implementation in many TB-endemic areas. This chapter provides a brief overview of the field with specific emphasis on common misperceptions, contentious issues and pragmatic strategies to improve implementation.

KEYWORDS: Preventive therapy, prophylaxis, screening, tuberculosis

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Global implementation of the DOTS (directly observed treatment, short course) strategy facilitated massive scale-up of diagnostic and treatment services for tuberculosis (TB) over the past 20 years. Estimated mortality rates fell by more than 30% during this period, with improved epidemiological outcomes in most regions of the world [1, 2]. However, the impact in the poorest countries and in areas affected by HIV infection has been limited, and the exclusive focus on sputum smear-positive disease essentially excluded children from care. In addition, the emergence of drug-resistant (DR)-TB poses a major threat, and requires novel approaches to enhance early case detection and rapid initiation of appropriate therapy [1, 2].

The high infection pressure that exists in TB-endemic areas, where ongoing transmission is sustained by high patient numbers and prolonged diagnostic and treatment delay, has only recently been appreciated [3, 4]. Annual risk of *Mycobacterium tuberculosis* infection studies, traditionally used to assess infection pressure within communities, are severely limited by the age restriction imposed. The use of primary school children in these surveys introduces significant selection bias, since their limited social contact is not representative of the community at large. This may result in a gross underestimation of the infection pressure experienced by adolescents and adults within the same community, especially among those with high-risk social behaviour.

Unfortunately, it is impossible to determine the true infection pressure within these high-risk subpopulations in the absence of a tool to measure re-infection.

The risk of re-infection has relevance, since it determines the ability to eradicate the pool of latent infection and the duration of benefit derived from treating latent infection. COMSTOCK *et al.* [5] achieved long lasting benefit from community-wide preventive therapy among the Inuit population in northern Canada by eliminating both the pool of latent infection and terminating ongoing transmission (by treating all cases with TB disease). The fact that all TB cases were removed from the community at the same time that the pool of latent infection was eradicated (by population-wide use of preventive therapy) explains its long-lasting "protective effect". Replicating these findings in modern high-incidence settings seems impossible, since constant mixing with other high-incidence populations would facilitate re-infection, despite eradicating latent infection from the resident population. However, its value to assist TB eradication in isolated populations and areas with limited transmission is well established.

Previous *M. tuberculosis* infection does offer some protection against future disease: a recent retrospective analysis of disease risk in previously infected (and otherwise healthy) *versus* uninfected adults indicated a 79% risk reduction among those with previous infection [6]. This identifies *M. tuberculosis*-uninfected individuals living in areas with a high TB-exposure risk as a vulnerable group, which is different to the risk perception in low-incidence areas where the risk of future re-activation disease is paramount. Despite the protection provided by previous infection, re-infection disease dominates the epidemic in areas with high infection pressure, as suggested by simultaneous infection with multiple strains and the fact that most adult cases harbour currently circulating strains (suggestive of recent infection/re-infection) [7]; 77% of TB recurrences have been attributed to re-infection [8]. In contrast to the risk reduction experienced by healthy individuals with *M. tuberculosis* infection, TB patients (who were previously unable to control *M. tuberculosis* infection) are at increased risk of TB recurrence [8]. This may reflect "selection" of the most vulnerable individuals, including contact with social networks that increase chances of repeat TB exposure.

Prevention of infection

M. tuberculosis is usually acquired by inhalation, following exposure to a source case with infectious TB. The risk of infection (primary or re-infection) is associated with the proximity and duration of contact, as well as the infectivity of the source case [9]. Sputum smear-positive cases pose the greatest transmission risk, although patients with sputum smear-negative forms of pulmonary TB also pose a transmission risk; with a relative risk (RR) of 0.24 (95% CI 0.20–0.30) [10]. Higher infection rates have been reported in child contacts of primary caregivers, such as mothers or grandmothers [11], which has particular relevance in HIV-affected communities where sputum smear-negative caregivers may pose a significant transmission risk to young and vulnerable children [12, 13].

It should be acknowledged that the most effective means to reduce the *M. tuberculosis* infection pressure within communities is to improve epidemic control. Early detection and effective treatment of infectious cases terminate transmission and reduce the high reproductive rate that sustains the TB epidemic. In TB-endemic areas, a high proportion of transmission occurs outside of the household, especially among older children and adults [14, 15], and within well-recognised "transmission hot-spots", such as drinking places (including informal shebeens), clinic waiting rooms and other congregate settings [16]. Creative infection control efforts within these areas may reduce the overall infection pressure within communities and enhance early case detection.

The emergence of DR-TB re-emphasised the importance of classical infection control measures. The World Health Organization (WHO) compiled an updated policy document on infection control [17], including strategies to reduce *M. tuberculosis* transmission within households, healthcare facilities and congregate settings. Unfortunately, less than a third of low- and middle-income countries surveyed during 2010 had a national TB infection control plan, and only 34 out

of 149 conducted any formal assessment [2]. Studies using guinea pigs to quantify the transmission risk in hospital wards demonstrated that effective treatment terminates transmission within days of initiation ([18] and personal communication: E. Nardell, Harvard School of Public Health, Boston, MA, USA). This increases the urgency to ensure early and effective treatment of all TB patients; it also supports the safety of home-based care programmes, even for patients with DR-TB, as long as treatment efficacy and adherence can be assured. For patients not on effective treatment, wearing a simple surgical face mask cuts transmission in half (56% reduction, 95% CI 33–70.5%) [19]. Transmission to close contacts often occurs prior to the identification of the source case and, therefore, careful screening of vulnerable contacts remains important. TB disease or *M. tuberculosis* infection in a young child also serves as marker of recent transmission and should trigger ''reverse contact tracing'' to identify the likely source case. Due to their limited social contact, the source case is usually identified among household members or other caregivers.

Prevention of progression to disease

Neonatal bacille Calmette–Guérin (BCG) vaccination does reduce the risk of severe TB disease in infancy, but the risk is not eliminated and the impact on adult-type disease is minimal [20]. Despite dedicated vaccine development efforts, TB will not become a vaccine-preventable disease in the near future [2]. In the absence of a fully protective vaccine, preventive therapy provides additional protection. ''Preventive therapy'' is an overarching term that includes any chemotherapeutic intervention that aims to prevent or reduce the risk of TB disease following TB exposure/infection.

''Treatment of latent infection'' implies documented subclinical infection, while the exact meaning of ''latency'' remains a topic of discussion and debate [21]. Subclinical lesions may represent recent infection (therefore, not yet ''latent'') or well-controlled asymptomatic (''truly latent'') infection. The rationale for the treatment of latent infection is to provide a limited course (either in duration or number of drugs) of treatment to sterilise existing subclinical lesions and reduce the risk of future disease. ''Primary prophylaxis'' implies therapy during a period of exposure, without proof of infection. Post-treatment or ''secondary prophylaxis'' aims to reduce the risk of TB recurrence following a course of TB treatment, evaluated in immunocompromised HIV-infected patients. With post-treatment prophylaxis, the observed reduction in TB recurrence is probably achieved by two mechanisms: 1) improved sterilisation of existing lesions; and 2) preventing re-infection events from becoming established (analogous to primary prophylaxis) [22].

Who should receive preventive therapy?

The dichotomy that exists between high-income countries where TB elimination is within reach and TB-endemic countries struggling to control the epidemic has been highlighted. Different levels of epidemic control influence the feasibility and rationale that guides the implementation of preventive therapy programmes. While young children, and HIV-infected and other immunocompromised individuals are universally prioritised, low-incidence settings often expand their focus to include recent immigrants, the socially destitute and the elderly, or may even target all infected individuals to try and eliminate the pool of latent infection. Table 1 summarises the priority groups and rationale for preventive therapy in different epidemiological settings.

Young and vulnerable children

Once infected, the risk of a child developing TB disease depends on the maturation and integrity of their immune system. Young age at the time of infection is a major risk factor for TB disease development. Natural history of disease studies conducted in the pre-chemotherapy era quantified the risk in different age groups as: 40–50% during infancy; 20–30% during the second year of life; 5% for the 2–5-year age group; and 2% for those 5–10 years of age [23]. Judging from autopsy studies [24], increased mortality of those aged under 5 years in TB-exposed households [25] and missed opportunities to prevent TB in children admitted to hospital with serious disease [26], successful

Table 1. Priority groups and rationale for preventive therapy in high- and low-incidence settings

Rationale	Target groups
High-incidence settings	
Huge disease burden with limited resources	Focus on the most vulnerable groups
Need to improve epidemic control	Young child contacts
Main aim:	HIV-infected patients
To reduce TB-related morbidity and mortality	Patients with pronounced immune compromise
	Other groups to consider
	Prisoners
	People in refugee camps
Low-incidence settings	
Limited disease burden with adequate resources	Focus on the most vulnerable groups
Need to move towards TB elimination	Young child contacts
Main aims:	HIV-infected patients
To reduce TB-related morbidity and mortality	Patients with pronounced immune compromise
To eliminate the ''pool of latent infection''	Other groups to consider
	Prisoners
	Refugees
	Asylum seekers
	Additional relevant pockets of infection
	Recent immigrants
	Old people
	TB contacts (all ages)

TB: tuberculosis.

implementation of pragmatic TB prevention strategies seems important to assist progress towards achieving the United Nations Millennium Development Goal (MDG) 4 targets [27].

HIV-infected individuals

The relative risk of developing TB in HIV-infected, compared with -uninfected, individuals is high, with incidence rate ratios exceeding 20 (20.6, 95% CI 15.4–27.5) [28]. Current CD4 cell count predicts TB risk, which is greatly reduced by antiretroviral therapy (ART), although the residual risk remains elevated (three to five times that of HIV-uninfected individuals) [29, 30]. Strategies that minimise the time adult patients spend below a CD4 threshold of 500 cells·mL^{-1} offers optimal protection [31], with higher, age-appropriate thresholds in children. In fact, ART should be initiated in all HIV-infected children within the first months of life to minimise mortality and TB risk [32].

In adults, the use of isoniazid preventive therapy (IPT) reduces TB risk but the benefit is restricted to those with a positive tuberculin skin test (TST) [33]. The operational challenges posed encouraged WHO to recommend standard IPT for all HIV-infected adults in settings where a TST is not feasible [34], although restricting the use of prolonged IPT courses to TST-positive individuals remains preferable [35]. The International Union Against Tuberculosis and Lung Disease (IUATLD) recently produced pragmatic guidance for TB/HIV management in resource-limited settings [36]. This emphasises the need for healthcare workers, especially those working in areas where TB exposure is likely, to know their HIV status, ensure optimal HIV management and use appropriate personal protection measures.

Other vulnerable groups

The risk increase associated with different types of immune compromise is highly variable and dependent on the likelihood of TB exposure/infection. Vulnerable groups include renal dialysis and transplant patients [37], and those receiving cancer chemotherapy [38] and long-term steroid [39] or tumour necrosis factor (TNF)-α treatment [40]. Immunomodulating conditions, such as diabetes mellitus and cigarette smoking, also increase TB risk. Insulin-dependent diabetes in children has been associated with a six- to seven-fold risk increase in TB-endemic areas [41], with

a two- to four-fold increase documented among adult patients with lifestyle-related diabetes [40]. Cigarette smoking doubles the TB risk, while smoking-related lung disease is associated with diagnostic delay and poor treatment outcome [40]. Circumstances associated with high levels of emotional stress and malnutrition combined with a high likelihood of TB exposure, as occurs in refugee or detention camps and prisons, also increase TB disease risk [42], but the impact of preventive strategies is poorly documented [43]. Healthcare workers and TB research assistants experience high rates of annual risk of *M. tuberculosis* infection (11.3% documented in healthcare workers) with increased risk of TB disease [44, 45]. Those with a negative TST or any form of immune compromise are particularly vulnerable [44, 46].

Contact screening and case finding

Contact screening represents an important opportunity for active case finding and TB education. All close contacts, irrespective of age, should be informed of their exposure and encouraged to return for formal evaluation should they develop symptoms suspicious of TB. The long delay between TB exposure and subsequent disease often obscures exposure–disease relationships and contacts may fail to appreciate that the risk window extends 2–5 years after exposure.

Lacking the capacity to perform a TST and/or chest radiograph often serves as a barrier to screening vulnerable contacts. Symptom-based screening offers a feasible alternative even in the most resource-limited settings, being safe and effective even in young children and in HIV-infected adults [47–49]. Figure 1 reflects the WHO symptom-based screening approach for child TB contacts [50]; all children with symptoms suggestive of HIV infection and/or TB disease should receive an HIV test, including those with an HIV-infected parent. HIV-infected individuals should be screened for TB exposure and/or disease at every healthcare contact, while repeated courses of IPT may be required in children with multiple exposures.

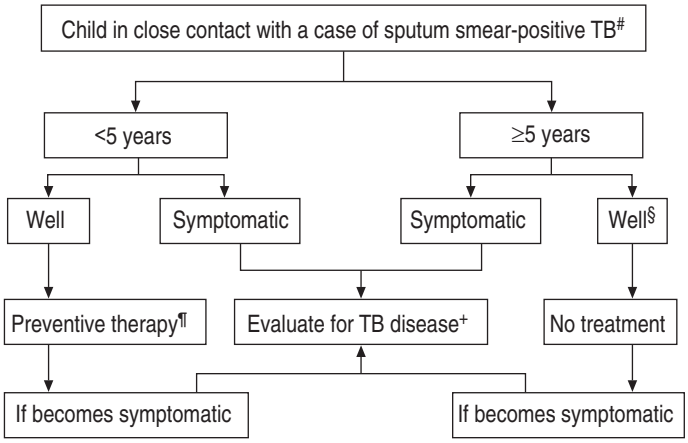

Figure 1. Suggested approach to contact management when chest radiograph and tuberculin skin testing are not readily available. The provision of preventive therapy to young and vulnerable children is not a ''one-time'' intervention: it should be provided after every documented tuberculosis (TB) exposure episode. #: also consider if the mother or primary caregiver has sputum smear-negative pulmonary TB; ¶: isoniazid monotherapy or combination therapy (refer to local guidelines); +: refer to local guidelines on diagnosis of TB disease; §: unless the child is HIV infected (in which case, isoniazid (10 mg·kg^{-1} daily for 6 months) is indicated). Reproduced and modified from [50] with permission from the publisher.

Preventive therapy options

Several regimens have been evaluated, including isoniazid (IPT) and rifampicin monotherapy, and combination therapy using isoniazid plus rifampicin or rifapentine and/or pyrazinamide, for durations of 2–12 months.

Isoniazid monotherapy

A Cochrane review of randomised controlled trials that included 73,375 HIV-uninfected adults and children found that IPT reduced the risk of TB disease by 60% (RR 0.40, 95% CI 0.31–0.52), with no significant difference between 6- and 12-month courses [51]. Combined evidence from US public health studies suggested a possible benefit from 9 compared with 6 months of post-exposure IPT, which explains the Centers for Disease Control and Prevention (CDC) recommendation

to use 9 months [52], compared with the 6 months advised by WHO [50]. A similar review of studies in HIV-infected individuals demonstrated a 36% overall reduction in TB disease risk (RR 0.64, 95% CI 0.51–0.81), but the benefit was restricted to those with a positive TST (RR 0.38, 95% CI 0.25–0.57) [33]. Prolonged or repeated IPT courses may also protect against re-infection disease. A randomised controlled trial comparing 6 to 36 months of IPT demonstrated enhanced efficacy in the 36-month arm, but this was again restricted to TST-positive individuals [53].

In HIV-infected children, the value of post-exposure prophylaxis and the need for ongoing exposure screening is universally acknowledged, and the potential value of secondary (post-TB treatment) prophylaxis is inferred from adult studies [54]. However, the potential value of routine (or pre-exposure) IPT in the most vulnerable young children remains an important point of controversy, due to contradictory study findings. The first randomised controlled trial conducted in children with limited ART access was terminated early by the data safety monitoring board, due to increased mortality in the placebo group [55]. Children were followed until 4 years after enrolment, and further analysis found that isoniazid was well tolerated with no increased risk of elevated liver enzymes, jaundice or fulminant liver failure in children taking ART and IPT in combination. After adjusting for age at follow-up, nutritional status and immunodeficiency at enrolment, isoniazid alone reduced the risk of TB disease by 78% (RR 0.22, 95% CI 0.09–0.53), ART alone by 67% (RR 0.32, 95% CI 0.07–1.55), and the combination of isoniazid and ART by 89% (RR 0.11, 95% CI 0.04–0.32) [56]. The second trial enrolled infants at a young age (3–4 months), excluded those with any TB exposure and provided early ART to all HIV-infected infants [57]. Participants were closely monitored for subsequent TB exposure, at which point they were taken off the study and provided with open-label isoniazid. No difference in TB disease or mortality could be detected, suggesting that routine IPT has little value if HIV-infected infants enter management programmes early, with meticulous TB exposure monitoring and provision of post-exposure IPT. However, the value of routine IPT as part of a comprehensive package of care in highly vulnerable HIV-exposed infants in TB-endemic settings, where exposure vigilance is likely to be suboptimal, remains unresolved.

Combination therapy

Rifamycins have strong sterilising activity, which could shorten preventive therapy regimens and improve efficacy in settings where isoniazid monoresistance is high. A prospective trial comparing IPT for 9 months *versus* 4 or 3 months of isoniazid and rifampicin reported no TB cases and minimal adverse events in children, while adherence was greatly improved with the shorter regimens [58]. Data from adult studies that included a large number of HIV-infected adults reported equivalence between 3 months of isoniazid and rifampicin *versus* isoniazid alone for 6–12 months [59], but this has not been endorsed by WHO. Pyrazinamide is another important sterilising drug and, used in combination with rifampicin for 2–3 months, it showed equal efficacy to isoniazid for 6–12 months, but results were marred by unacceptably high rates of hepatotoxicity in HIV-uninfected adults [60]. A novel strategy using 3 months of weekly long-acting rifapentine and isoniazid (12 doses in total) proved highly efficacious in adults and has been recommended by the CDC [61]. It is not recommended for people on ART, pregnant females or children less than 12 years of age, until more pharmacokinetic and safety data become available. A limitation of all rifampicin- and rifapentine-containing regimens is the interaction with protease inhibitor-containing ART treatment. This is reduced with rifabutin but its use in preventive therapy regimens has not been evaluated.

Adverse events

Severe adverse events are uncommon with the use of preventive therapy [62]. In general, children tolerate TB drugs better than adults, which may reflect differences in prevalence of other risk factors, such as alcohol use or underlying hepatic disease, and differences in pharmacokinetics. With isoniazid use, subclinical transient transaminase elevation is a frequent occurrence, but severe isoniazid-induced hepatitis is very rare. Hepatotoxicity is the major concern with the combined use of rifampicin and pyrazinamide being more common in HIV-uninfected adults [60]. Data in

children are limited, but standard first-line therapy is well tolerated and no hepatotoxicity was reported from trials that evaluated 2 months of rifampicin and pyrazinamide [63, 64]. Increased rates of possible hypersensitivity reactions were reported with the use of isoniazid and rifapentine [65].

Policy–practice gap

Despite the sound scientific basis underlying current recommendations and their enormous potential to reduce the burden of TB disease in children, a massive policy–practice gap exists and contact management is almost nonexistent in areas of high incidence [66]. Among HIV-infected patients newly enrolled in care, only 12% received IPT during 2010 [1]. Key implementation barriers underlying this policy–practice gap include: 1) ignorance regarding TB disease burdens in vulnerable individuals, especially young children; 2) concerns about reliable exclusion of TB disease and poor treatment adherence that may fuel the emergence of DR strains; 3) resource constraints and prioritisation; and 4) the absence of effective monitoring and evaluation systems.

Ignorance regarding the TB disease burden in young children reflects poor quantification at a global level and the focus on sputum smear-positive disease under the DOTS strategy. Appreciation of TB's contribution to morbidity and mortality of those aged under 5 years in TB-endemic areas is growing, and the need for pragmatic preventive therapy strategies has been recognised. Studies have demonstrated that TB disease can be reliably excluded in vulnerable populations, such as children and HIV-infected adults, with pragmatic symptom-based approaches. Concern about "fuelling DR-TB" seems unfounded, especially in young children who tend to develop paucibacillary disease with reduced risk of both acquiring drug resistance and transmitting this within the community. It is important to exclude adults with cavitary lung disease and high bacillary loads, but this can be achieved with pragmatic screening approaches and regular follow-up. Simple systems can be implemented to minimise risk and evidence suggests that DR disease is not increased in well-functioning programmes. There is a need for basic training and capacity building, but this is perfectly feasible with adequate political commitment and resource allocation. Apart from preventing severe disease in vulnerable individuals, preventive therapy is also cost-effective from a TB control perspective, if high rates of implementation can be achieved [67]. However, effective monitoring and evaluation systems are required, since measurement drives implementation. Table 2 summarises the barriers to the provision of TB preventive therapy in high-incidence settings.

Drug-resistant TB

Guidance for preventive therapy of DR source case contacts is hampered by the absence of empirical evidence. However, useful guidance can be provided from clinical experience and available data.

Monoresistance

Regular surveillance for isoniazid resistance is important, as it is often the first step in the development of multidrug-resistant (MDR)-TB. Variable levels of resistance are associated with specific mutations, low-level resistance with an *inhA* promoter region mutation and high-level resistance with *katG* gene mutations [68], which explains why high-dose IPT may still provide some protection after exposure to a DR source case. With documented exposure to an isoniazid-monoresistant source case, rifampicin monotherapy for 4 months is advised. Rifampicin monoresistance is less common, but it does occur and is on the increase [69]. Following exposure to a rifampicin-monoresistant source case, standard IPT should be sufficient. However, rifampicin resistance is usually managed as MDR-TB, since rapid drug-susceptibility testing (DST) methods such as GeneXpert® (Cepheid, Sunnyvale, CA, USA) do not test for isoniazid resistance, which poses problems for the provision of effective preventive therapy.

Table 2. Barriers to the provision of tuberculosis (TB) preventive therapy to the most vulnerable groups, in high-incidence settings

Risk group	Barriers	Comment
Young child contacts	Failure to appreciate the risk	Young children, especially those <2 years of age, are at high risk of developing TB disease (including disseminated disease and TB meningitis) following exposure/infection
	Perception that young children are protected by BCG vaccination	BCG provides only partial protection and disease risks remain high
	Lack of political will to implement	Despite the absence of accurate disease burden data, studies from multiple sites in sub-Saharan Africa indicate high TB-related morbidity and mortality in young children
	Child TB does not have a ''face''	
	Absent monitoring and evaluation	Implementation is driven by monitoring and evaluation
	Perceived inability to exclude TB disease	Pragmatic symptom-based screening is safe and feasible in all settings
	Perceived lack of resources	Some additional resources would be required, but mostly only increased training and supervision
	Fear of ''creating'' drug resistance	This is not an issue in young children
	Poor adherence	Parental education/counselling and shorter courses increase adherence
HIV-infected patients	Poor HIV services in some settings	ART is the most effective intervention to reduce TB risk
	Not seen as a priority	Significant additional risk reduction possible
	Fear of ''creating'' drug resistance	Within a well-functioning HIV care programme, the risk is low
	Poor adherence	This requires patient education and follow-up
	Confusion around guidance in children	There is no confusion around the need for constant exposure vigilance and the provision of post-exposure preventive therapy with every occurrence
Others with immune compromise	Not a well-defined group	Highly variable vulnerability and exposure risk
	Few internal protocols	Need for protocols relating to specific patient groups at high risk of reactivation disease *e.g.* those receiving immunosuppressive therapy
	Small isolated constituencies	Formulation of international generic standards of care for specific risk groups would be helpful

BCG: bacille Calmette–Guérin; ART: antiretroviral therapy.

Multidrug resistance

The evidence base for the provision of preventive therapy to MDR-TB contacts is limited; a systematic review found no randomised trials and only two observational studies on preventive therapy in MDR-TB contacts [70]. In general, second-line drugs are also more toxic. In clinical practice, preventive therapy has been restricted to the most vulnerable contacts, providing two drugs to which the organism is susceptible (or naïve) for at least 6 months, with regular follow-up for 1–2 years [71]. Using this strategy, a prospective cohort study in children demonstrated a significant reduction in TB disease among those receiving preventive therapy (RR 0.2, 95% CI 0.04–0.94) [72]. Later-generation quinolones demonstrate similar potency to rifampicin and 4–6 months of monotherapy should conceivably be adequate, if quinolone resistance is reliably excluded. However, recent European Center for Disease Prevention and Control (ECDC) guidance concluded that due to limited evidence, more research is warranted before any firm recommendations can be made [73, 74], supporting current WHO guidance [75].

Conclusions

In areas of high incidence, improved epidemic control through early case finding and treatment initiation remains the key focus, but creative infection control measures should be considered. Limited resources and high rates of ongoing transmission justify restriction of TB preventive therapy to the most vulnerable, which includes child contacts and HIV-infected individuals. Although efficacy and safety are well established and pragmatic strategies have been developed, implementation remains woefully lacking, especially in vulnerable young children.

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Statement of Interest

None declared.

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Chapter 8

TB drug resistance in high-incidence countries

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SUMMARY: The burgeoning epidemic of drug-resistant (DR)-TB threatens to destabilise TB control in high-burden settings, particularly in Africa. From a cost perspective, drug resistance consumes a disproportionate amount of national TB programme budgets in resource-poor settings and is not sustainable. With the roll-out of new technologies, such as the Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) assay, there will need to be an appropriate scale-up of treatment services throughout high-burden settings. Compared with intermediate and low-burden settings, DR-TB in high-burden settings is characterised by an increase in the overall case burden, lack of access to first- and second-line susceptibility testing, limited or no access to second-line drugs, higher default rates and a poorer prognosis. Of concern is the increasing number of extensively drug-resistant (XDR)-TB cases, and therapeutically destitute XDR-TB treatment failures, which pose a major logistical and ethical dilemma in high-burden settings. In this chapter, we review the epidemiology, diagnosis, management and prognosis of DR-TB with a specific emphasis on high-burden settings.

KEYWORDS: Diagnosis, drug-resistant tuberculosis, epidemeology, high burden, prognosis, resource-poor

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Drug-resistant (DR)-tuberculosis (TB) is an important public health problem in many resource-poor settings [1, 2]. In contrast to intermediate and low-burden settings, the overall prevalence and rates of multidrug-resistant (MDR)-TB are increasing in many high-burden countries. In these countries, default rates are high, and compared with intermediate- and low-burden settings, unfavourable outcomes are more common [3]. The disease afflicts economically active young adults and has a substantial mortality rate. DR-TB also threatens to destabilise control programmes in high-burden settings. For example, in South Africa, despite DR-TB comprising less than 2% of the total caseload, it consumes almost 30% of the total national TB programme budget, and almost 60% of the annual TB drug budget (A. Pooran and K. Dheda; unpublished data). Clearly, this is not sustainable. In this chapter, we review the epidemiology diagnosis management and prognosis of MDR-TB (resistance to rifampicin and isoniazid) and extensively drug-resistant (XDR)-TB (resistance to rifampicin, isoniazid, any fluoroquinolone and

any one of the three injectable agents, *i.e.* amikacin, kanamycin or capreomycin), with a particular emphasis on high-burden settings.

Epidemiology

It is estimated that approximately 440,000 cases of MDR-TB were diagnosed in 2008 [4–8]. Of these, it is estimated that approximately 40,000 had XDR-TB. Of the estimated 440,000 cases, 360,000 were new and relapsed cases, and approximately 94,000 were persons previously treated for TB. Thus, the majority of MDR-TB cases were not due to acquired resistance. This is an important point to appreciate because cost-saving strategies that target diagnostic tests to only patients with risk factors for DR-TB will miss a substantial number of MDR-TB cases, and contribute to the amplification of resistance.

Most of the burden of MDR-TB lies within the 27 high-burden countries. However, India and China are responsible for nearly 50% of the global MDR-TB burden, followed by Russia at just under 10% [4–8]. MDR-TB is estimated to have caused approximately 150,000 deaths in 2010 [4–8]. The proportion of patients with MDR-TB in new and previously diagnosed cases is shown in chapter 2 of this issue [9] and was previously reported by the World Health Organization (WHO) [10]. These are global estimates based on periodic surveys carried out in various countries and analysed by the Global Project on Anti-tuberculosis Drug-resistance Surveillance. Given that no regular surveys are undertaken in the highest burden countries, it is difficult to ascertain whether rates of MDR-TB are increasing or decreasing. While the incidence of MDR-TB is falling in some parts of the world, in other countries such as Peru, South Korea and Botswana the incidence of MDR-TB is increasing [11]. In South Africa, in a nationwide survey performed in 2002, the proportion of MDR-TB in new and retreatment cases was estimated at 1.8 and 6.7%, respectively [12]. Thus, in 2002, 3.1% of all TB cases had MDR-TB. In a nationwide survey in 2008, 9.6% of all cases were shown to have MDR-TB, thus representing a dramatic three-fold increase since 2002 [12]. The molecular epidemiology of DR-TB in South Africa mirrors what is happening in many other high-burden settings and countries in Africa. In South Africa, approximately 80% of MDR-TB is due to primary transmission [12]. By contrast, it was recently shown in several provinces in South Africa that XDR-TB is mainly acquired [13]. However, increasing numbers of clusters suggest primary transmission being observed as the epidemic progresses. Primary transmission of DR-TB is fuelled by the HIV pandemic in Africa. Superimposed upon this are extended outbreaks, as described in Tugela Ferry in 2006 [11]. Furthermore, the dominant TB strains are variable, even in adjacent provinces [12]. Thus, the clinical and molecular epidemiology of DR-TB, even within one country, is complex. Of concern is that routine culture and drug-susceptibility testing (DST) occurs in only 22% of countries worldwide [2]. It is alarming that in several countries of the former Soviet Union, more than 12% of new cases and more than 50% of previously treated cases are MDR-TB. Thus, there is a burgeoning burden of DR-TB in many intermediate and high-burden countries.

It is important to note that there is a lack of data from many countries in Africa and periodic surveys are almost non-existent. Even when surveys are performed it is only on a limited basis geographically, they often exclude patients in the private sector, and they often do not represent a diagnosis made using new technologies such as GeneXpert® (Cepheid, Sunnyvale, CA, USA). Thus, there are severe limitations to the current data. Nevertheless, recent opportunistic surveys from several African countries indicate MDR prevalence rates varying from 10–26% [1]. Additional well-conducted surveys from Africa are therefore urgently needed. Detailed reviews about the molecular epidemiology of TB in high-burden settings have recently been published [1, 12].

Diagnosis of DR-TB in high-incidence countries

Tests for DR-TB can aid the early selection of effective therapy, thereby helping reduce otherwise frequent unfavourable outcomes such as treatment failure or death [2, 3], and person-to-person transmission [14]. In 2008, however, due to limited laboratory capacity, only 1% of new TB cases

in the 27 high-burden MDR-TB countries received DST and, of the approximate half a million global MDR-TB cases, only about 6% are thought to have been correctly diagnosed [15, 16]. There is, thus, an urgent need for new tests for DR-TB suited to high TB burden countries [17]. Here we provide a brief overview of conventional phenotypic tests as well as new molecular tools for the diagnosis of drug resistance, which promise to reduce diagnostic delay and permit the earlier initiation of appropriate anti-TB therapy (table 1). Tests for which there is limited or poor evidence to justify their use, such as the Ibis mass spectrometry platform [37, 38], as well as other non-routine tests for drug resistance are described elsewhere [18, 24, 39–42].

Culture-based tests for DR-TB

Conventional DST detects the absence (indicating susceptibility) or presence (indicating resistance) of *Mycobacterium tuberculosis* growth in the presence of specific anti-TB drugs. Most culture-based tests involve indirect DST, in which the drug-containing medium is inoculated with a pure culture isolate grown from the original patient specimen. Solid agar methods, such as the agar proportion method, are the most accurate and generally serve as the diagnostic gold standard [24]; however, liquid culture systems, such as the Bactec™ MGIT™ 960 system (Becton-Dickinson, Sparks, MD, USA), have been shown to mostly possess equivalent performance and are WHO recommended [43]. More recently, rapid growth- and microscopy-based DST, such as the microscopy observed drug susceptibility (MODS) method and thin layer agar (TLA) technique, have been developed and are WHO endorsed [25, 44]. The drawbacks of these techniques include a relatively high level of technical complexity, a still suboptimal time-to-diagnosis (~10 days with MODS) and the inability to multiplex (*i.e.* having to perform a separate assay for each drug).

New molecular methods for the detection of drug resistance

Recently, several new genotypic tests for drug resistance, which rely on the amplification and detection of nucleic acid mutations strongly associated with resistance, have been developed (fig. 1). These include the WHO-approved Xpert® MTB/RIF [26–28, 45], INNO® LiPA RifTB (Innogenetics, Ghent, Belgium), Genotype™ MTBDR*plus*® (Hain Lifescience GmbH, Nehren, Germany) [19, 31], and Genotype™ MTBDR*sl*® assays (Hain Lifescience) (which is currently under review by the WHO), and can, in theory, provide results within hours.

Xpert® MTB/RIF

The key advantage of Xpert® MTB/RIF, which is primarily a test for the presence of *M. tuberculosis*, is that the detection of mutations associated with rifampicin resistance occurs simultaneously as part of the primary assay [46]. Hence, in countries such as South Africa, which are introducing Xpert® MTB/RIF as the upfront test for all patients with suspected TB, all individuals will automatically also receive rifampicin susceptibility testing, often within 1 day of specimen collection [27]. Resistance to this drug serves as a good surrogate marker for MDR-TB in a population with a high prevalence of drug resistance [27, 28]. However, even in countries like South Africa with an overall MDR-TB prevalence of 5–10%, further confirmatory testing (either in the form of a line probe assay or a conventional culture-based test) may be required [47] and is recommended by South African national guidelines [48]. This is because Xpert® MTB/RIF's positive predictive value (PPV) for rifampicin resistance is, even with a specificity of ~98%, only 70–85% [49]. In conjunction with the Foundation for Innovative and New Diagnostics, the manufacturer has since attempted to optimise the assay to correct for this issue; however, data about its new performance is currently limited [50], and further research is required before the need for a confirmatory DST in patients at a low risk of MDR-TB can be confidently eliminated.

Relevant to MDR-TB, where in some settings 80% of cases are caused by person-to-person transmission [12], Xpert® MTB/RIF results correlate with bacterial load markers, such as smear grade [26], and the quantitative information generated by this assay (known as cycle threshold (C_T) values) can be used to identify individuals who probably represent the greatest infectious risk (a recent study showed that C_T >31.8 had moderately good rule-out value for smear-positivity [29]).

Table 1. Culture- and molecular-based tests for drug resistance in *Mycobacterium tuberculosis*

	Test description	First-line DST[#]	Second-line DST[¶]	Advantages	Limitations	Commercial versions
Culture-based methods						
Solid agar (Middlebrook or Lowenstein–Jensen) techniques [18–20]	The most widespread solid agar technique (the agar proportion method), involves detecting the presence or absence of growth in the presence of the anti-TB drug of interest. If the agar plate containing the drug has ⩾1% the number of colony forming units as a plate without the drug, it is classified as resistant.	Yes	Yes	Highly accurate (considered the ''gold standard'' of DST) Relatively inexpensive WHO approved	Slow time-to-result (3–4 months) Some standardisation challenges with regard to second-line DST	
Automated liquid techniques [21–23]	Decontaminated sputum is used to inoculate a liquid media-containing tube, which is continuously monitored for growth *via*, for example, a sensor which detects oxygen consumption.	Yes	Yes	Highly accurate Several days faster than solid agar DST WHO approved	Results from raw sputum can still take 2–3 months Higher rates of contamination than solid agar DST [21–23]	Bactec™ MGIT™ 960 system and SIRE™ and PZA kits (Becton–Dickinson) VersaTrek/ESP (Difco-Accumed International) BacT/Alert3D (bioMerieux)
Non-automated microcolony culture techniques (MODS or TLA) [24, 25]	Drug-free or drug-containing media is inoculated with specimens from patients and examined microscopically for growth.	Yes	No	Excellent accuracy for first-line DST Generally rapid (mean turnaround time for MODS and TLA of 10 and 11 days, respectively) Can be performed directly on specimens Relatively inexpensive and non-proprietary Approved by the WHO	Sensitivity for second-line drugs appears suboptimal; however, more evidence is needed May not be suited to high NTM settings Require significant technical expertise Not widely available	TB MODS Kit® (Hardy Diagnostics)
Molecular methods						
Fully automated nucleic acid amplification assay (Xpert® MTB/RIF) [26–30]	Liquefied sputum is added to a cartridge and inserted into the GeneXpert® machine. A run is typically completed within 2 hours.	Yes[+]	No	Rapid (median time to result of 0–1 days) Can be performed directly on specimens DST occurs simultaneously with TB detection Closed cartridge system, which minimises cross-contamination and technical expertise required	Only provides information regarding rifampicin resistance Patients with a low pre-test probability of MDR-TB who are positive for rifampicin resistance may require further investigation Relatively costly	GeneXpert® MTB/RIF (Cepheid)

Table 1. Continued.

	Test description	First-line DST[#]	Second-line DST[¶]	Advantages	Limitations	Commercial versions
Line probe assays [31–36]	PCR amplicons are passed over a lateral flow strip containing probes for specific mutations. Bound amplicons and mutations are subsequently detected colorimetrically.	Yes[§]	Yes[§]	Rapid (time to result of 2 days) Can be performed directly on specimens	Sensitivity generally lower when performed on smear-negative samples Relatively costly An open system potentially vulnerable to cross-contamination	MTBDR*plus*® and MTBDR*sl*® (both Hain Lifesciences GmbH) INNO® LiPA RifTB (Innogenetics)

DST: drug-susceptibility testing; TB: tuberculosis; WHO: World Health Organization; PZA: pyrazinamide; MODS: microscopic observation drug susceptibility; TLA: thin layer agar; NTM: non-tuberculosis mycobacteria; MDR: multidrug-resistant. [#]: defined as resistance to rifampicin and isoniazid. [¶]: defined as resistance to rifampicin, isoniazid, any fluoroquinolone (ofloxacin, levofloxacin, moxifloxacin), and any of the aminoglycosides (amikacin, kanamycin or capreomycin). [+]: Xpert® MTB/RIF detects *rpoB* mutations, which are associated with resistance to rifampicin. [§]: INNO® LiPA RifTB detects only *rpoB*-mutations associated with rifampicin resistance. MTBDR*plus*® additionally detects *katG* and *inhA* mutations associated with isoniazid resistance. MTBDR*sl*® detects mutations within the *rrs*, *gyrA* and *embB* genes. The details of each of the manufacturers listed are as follows. Becton-Dickinson: Sparks, MD, USA; Difco-Accumed International: Westlake, OH, USA; bioMerieux Inc.: Durham, NC, USA; Hardy Diagnostics: Santa Maria, CA, USA; Cepheid: Sunnyvale, CA, USA; Hain Lifesciences GmbH: Nehren, Germany; Innogenetics: Ghent, Belgium.

Line probe assays

The performance of the line probe assays, a type of nucleic acid amplification assay in which the products are hybridised onto a lateral flow strip, for first-line (MTBDR*plus*®) [32] or second-line (MTBDR*sl*®) [33–35] drugs is generally good (sensitivity of ~98% for MTBDR*plus*®, and sensitivities of 70–95% for MTBDR*sl*®, with overall good specificity for each assay of ~99%) when performed on smear-positive specimens, although it is improved further when pure culture isolates are used (fig. 1). However, these assays still do not have sufficient accuracy, especially for second-line drugs and injectable agents, and have relatively high indeterminate rates in smear-negative clinical samples. There is therefore a clinical need for new rapid tests in this area, before they can completely replace culture-based methodologies. A newer version of the MTBDR*plus*® assay has recently been launched and may have improved performance in smear-negative TB cases [51], although further data are awaited. The suboptimal sensitivity of MTBDR*sl*® for the second-line injectable drugs means that a negative test does not necessarily rule out resistance to second-line drugs [33], and that culture-based DST would still be required. The key advantages of these tests are their rapidity and the high confidence that can be placed in a positive result. Their key disadvantage is their technical complexity.

Conclusion

Molecular DST has matured to a point where, with the proper expertise and infrastructure, such tests are technically feasible in high-incidence countries. These methods perform well and, although they are not as accurate as culture-based phenotypic methods, they are able to provide results within hours or a few days, thereby potentially greatly enhancing patient care. They are also becoming increasingly automated and simpler to perform. Nevertheless, these technologies are relatively expensive and simple DST technologies suited to the point-of-care are needed [52].

Management of MDR-TB and XDR-TB

Treatment of MDR-TB is complex and uses toxic drugs that must be administered for a much longer duration than for drug-susceptible TB patients, and with a lower likelihood of treatment success [53].

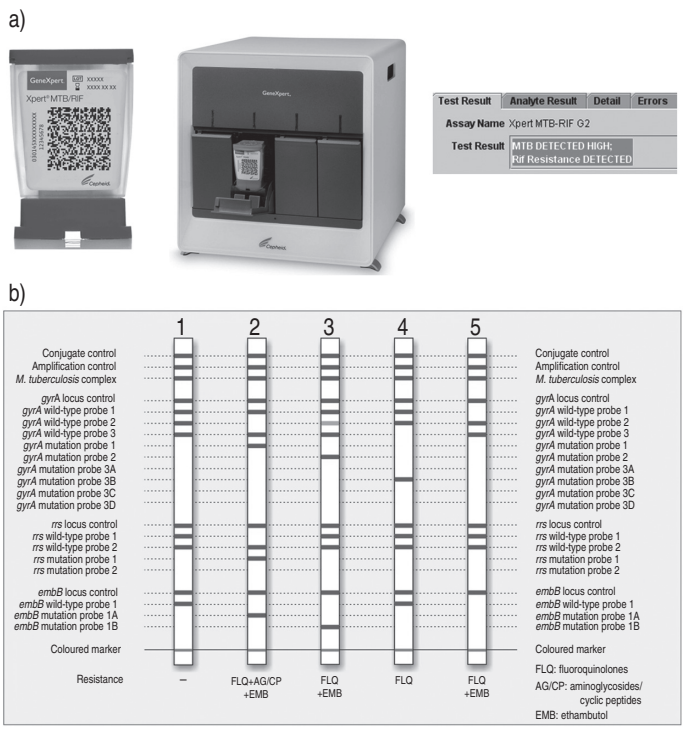

Figure 1. New rapid tests for detecting mutations associated with drug-resistant tuberculosis (TB). a) The Xpert® MTB/RIF test (Cepheid, Sunnyvale, CA, USA), detects rifampicin resistance simultaneously with *Mycobacterium tuberculosis*. The test is largely automated and endorsed by the World Health Organization. Sputum treated with sample buffer is added to a single-use MTB/RIF cartridge (left panel), prior to loading into a GeneXpert® machine (middle panel; a four-module GeneXpert® machine is depicted), before reading the result about 2 hours later (right panel; information regarding TB status, bacterial load and rifampicin susceptibility is shown). b) The Genotype™ MTBDR*sl* assay (Hain Lifescience GmbH, Nehren, Germany), which detects mutations in the *rrs*, *gyrA* and *embB* genes (associated with resistance to the aminoglycosides, fluroquinolones and ethambutol, respectively), is shown. Nucleic acid amplification products are separated *via* lateral flow onto a strip containing probes corresponding to specific mutations. Upon binding to a probe, a colorimetric reaction occurs and the presence or absence of a mutation is visualised. *M. tuberculosis*: *Mycobacterium tuberculosis*. Images are courtesy of the respective manufacturers.

There are several challenges in managing a patient with MDR-TB. First, it is essential to correctly diagnose MDR-TB otherwise the patient will erroneously receive potentially toxic drugs. An effective and tolerable regimen should be selected and all effort must be made to keep the patient on treatment until the end of the course, paying particular attention to managing the side-effects of treatment, which if not effectively dealt with, may lead to the patient interrupting treatment.

Use of drugs to which the strain is reportedly susceptible is more effective when compared with their use regardless of susceptibility patterns [54]. The choice of drug would depend on DST of the strain isolated from the patient or close contact with MDR-TB, previous use of the drug in the patient and frequency of use of the drug or documented background drug resistance in the setting [54].

In the intensive phase, a regimen with at least four drugs is recommended. An injectable agent in the form of kanamycin, amikacin or capreomycin should be administered unless there is a contra-indication such as advanced age or renal failure. No second-line parenteral agent is superior to any other. Because of its lower cost, kanamycin is preferable but amikacin can be used instead of kanamycin. Capreomycin is recommended if there is hearing impairment. The use of streptomycin in MDR-TB is not advisable given the greater likelihood of ototoxicity and the frequent occurrence of resistance to it among MDR-TB patients [54]. Fluoroquinolones should always be used unless there are contra-indications. Later-generation fluoroquinolones such as moxifloxacin, levofloxacin or gatifloxacin are preferred. The inappropriate use of fluoroquinolones may drive resistance to this drug [55]. Among the bacteriostatic drugs the association with cure is highest for ethionamide or prothionamide compared with cycloserine (or terizidone) or para-amino salicylic (PAS) acid [54]. PAS should probably only be used in the treatment of MDR-TB if the patient cannot tolerate ethionamide or prothionamide, or cycloserine. The addition of pyrazinamide to a four-drug regimen is recommended.

An intensive phase of $\geqslant$8 months' duration is recommended and a total duration of $\geqslant$20 months' treatment is advisable in patients without any previous MDR-TB treatment [54]. Patients who have had previous MDR-TB treatment should have $\geqslant$24 months' treatment.

The treatment of XDR-TB is problematic as the data on successful treatment regimens is poor. Capreomycin and PAS are the foundations of treatment when they have not been used before. A later-generation fluoroquinolone is recommended despite *in vitro* resistance as regimens containing a fluoroquinolone have a higher success rate than those that don't [3]. Ethionamide or prothionamide should be used if DST still shows the organism is susceptible to them. Drugs from WHO Group 5 (clofazamine, linezolid, thiocetazone, high-dose isoniazid, meropenem, imipenem, co-amoxyclavulic acid, clarithromycin, azithromycin, roxithromycin and thioridazine) will need to be used depending on funding and availability [4, 56]. The efficacy of linezolid in the treatment of complicated DR-TB has recently been reviewed [57, 58]. There is also recent preliminary data on the efficacy of the meropenem–clavulante combination in XDR-TB [59]. The exact number of drugs used to treat XDR-TB is unknown but most patients will receive five or six drugs. The intensive phase with capreomycin should be at least 8 months and many patients will receive 12 months of therapy. The duration of treatment should be 24–30 months, and if there is residual localised fibrocavitatory disease, surgery should be considered. Table 2 shows the main side-effects encountered in treating M(X)DR-TB and the drugs responsible for them.

In addition to monitoring sputum cultures it is important to monitor serum creatinine and potassium monthly while on an injectable and weekly while on capreomycin. Thyroid-stimulating hormone (TSH) should also be monitored between month 6 and 9 while on ethionamide, prothionamide or pyrazinamide. Haemoglobin, platelets and white cell count should be monitored monthly while on linezolid. Highly active antiretroviral therapy (HAART) is recommended in all patients who are HIV-infected irrespective of CD4 count within the first 8 weeks of anti-TB treatment [54]. With regard to where patients with MDR-TB should be managed it is recommended that they should be treated using mainly ambulatory care. This is more cost-effective than hospitalisation. However, there may be some significant barriers to accessing clinic care.

Surgical resection plays an important role in the management of patients with M(X)DR-TB. Patients must have adequate cardio-pulmonary function and essentially unilateral disease. In assessing a patient for surgery, a computed tomography (CT) chest scan is required to assess the extent of disease. Spirometry and a 6-minute walk test (6MWT) are performed, and in patients with borderline lung function, a lung perfusion scan can help to decide on operability. The indications for surgery are: 1) failed chemotherapy with an XDR-TB regimen; 2) patients with previous MDR-TB who relapse in the same site; 3) all XDR-TB patients who have sputum converted; 4) patients with intolerable side-effects whose sputum has converted to negative; and 5) specific requests for surgery from a patient. Residual cavitation or fibrosis after initial successful treatment of MDR-TB is not, on its own, an indication for surgery.

Table 2. Side-effects of multidrug-resistant (MDR)- and extensively drug-resistant (XDR)-tuberculosis treatment

Side-effect	Drug
Nausea and vomiting	Ethionamide, ethambutol, fluroquinolones, pyrazinamide, PAS, clofazimine
Diarrhoea and abdominal pain	PAS
Hearing loss	Amikacin, kanamycin, capreomycin
Electrolyte disturbances	Capreomycin
Renal impairment	Amikacin, kanamycin, capreomycin
Peripheral neuropathy	High-dose isoniazid, linezolid, ethambutol
Joint and muscle pain	Fluroquinolones, pyrazinamide
Fatigue and hair loss	Ethionamide, PAS
Depression and psychosis	Terizidone, high-dose isoniazid, fluroquinolones
Insomnia	Fluroquinolones

PAS: para-amino salicyclic acid.

New drug treatments

The current drugs available for the treatment of MDR-TB are inadequate. There are concerns about currently available agents and regimens, and these include increasing levels of mycobacterial resistance, poor tolerability and unacceptable side-effect profiles. In developing countries there is limited access to essential drugs such as fluoroquinolones, and extended treatment periods of 18 months and longer impact heavily on patient retention and compliance.

There is currently a strong international push to develop new anti-TB drugs [60]. The ideal new prototype agent should be able to treat both drug-sensitive and drug-resistant organisms alike, have a favourable side-effect profile, be cheap to manufacture, not exhibit interactions with antiretroviral drugs and in its action should potentially shorten the duration of anti-TB treatment.

New anti-TB drugs undergoing clinical testing are listed in table 3. While the ultimate goal in anti-TB therapy is to engineer an entirely new combination regimen which could be used to treat both drug-sensitive and -resistant isolates alike, the reality is that newer agents will be introduced either as add-on therapy to existing MDR-TB regimens or in combination with existing agents such as moxifloxacin and pyrazinamide.

TMC207 (Bedaquiline) is a diarylquinoline compound with a novel method of action. TMC207 specifically inhibits mycobacterial adenosine triphosphate (ATP) synthase [61]. *In vitro*, TMC207 inhibits both drug-sensitive and -resistant mycobacterial isolates and exhibits a synergistic mode of action with pyrazinamide in the murine model. In a phase 2 clinical trial, the addition of TMC207 to standardised therapy for MDR-TB reduced the time to conversion to negative sputum culture *versus* placebo and significantly increased the proportion of patients with negative sputum cultures after 8 weeks [62]. This is the first evidence that this drug may be considered as a potential new treatment for MDR-TB. The results of the rigorously conducted 2-year follow-up of these patients was recently presented [63]. During this period, the emergence of new drug resistance was substantial, and it appears that the onset of acquired resistance was reduced by the concurrent administration of TMC207. This indicates that, like ethambutol in the standard regimen, TMC207 may have a potential role in protecting companion drugs against acquired drug resistance.

OPC67683 (Delamanid), a member of the nitroimido-oxazole subclass, has been tested in phase 2b [64] studies and in MDR-TB [65], and has shown an increase in sputum culture conversion at 8 weeks in the context of add-on therapy to a MDR-TB background regimen.

Immunotherapeutic approaches to DR-TB

DR-TB treatment regimens are complex, toxic and expensive. New drug development is slow and therefore novel therapeutic strategies are needed. Immunotherapeutic approaches are promising and warrant increased research efforts [66, 67]. Important potential roles for adjunctive immunotherapies in MDR- and XDR-TB include: improving cure rates and decreasing time to culture conversion; decreasing the tissue damage associated with non-productive host immune responses; and improving overall health status by mitigating systemic impacts of long-standing chronic

Table 3. New anti-tuberculosis (TB) agents with potential use in multidrug-resistant (MDR)-TB

Compound name	Clinical development phase	Comments
TMC-207 (Bedaquiline)	3	Effective in MDR
OPC-67683 (Delamanid)	3	Effective in MDR
SQ-109	2	May prevent drug resistance
PA-824	2	Bactericidal; may treat latent TB infection
PNU-100480 (Sutezolid)	2	May be effective in MDR
AZD-5847	Early 2	New linezolid derivative

inflammation. In addition, as a number of potentially useful cytokines and other immunomodulatory agents are already in clinical use for other conditions, the long regulatory processes associated with new drug development could be bypassed, and therapies for DR-TB could move directly into phase IIb and III clinical trials.

Immunotherapies for DR-TB can be considered in three main groups: 1) immunomodulatory agents to induce a favourable immune response *e.g.* anti-interleukin (IL)-4 to potentially attenuate T-helper cell (Th) type 2 responses thereby altering the Th1/Th2 balance towards Th1; 2) immunosuppressive agents, *e.g.* thalidomide (decreases tumour necrosis factor (TNF)-α), to suppress deleterious inflammation thereby limiting tissue damage or potentially improving the access and/or susceptibility to TB drugs; and 3) adjunctive cytokine therapy, *e.g.* interferon (IFN)-γ, to enhance the mycobacteriocidal activity of effector immune cells [66, 67]. Table 4 shows immunotherapies that have been evaluated for use in patients with TB.

Despite the ready availability of several immunotherapies, a number of barriers to the use of immunotherapy for DR-TB remain [67, 92]. To date, the success of adjunctive immunotherapy for TB has been variable. Studies (mainly in DS-TB) have either shown: benefits in very small numbers of patients or only murine models, *e.g.* mesenchymal stem cells (MSC) [67], intravenous immunoglobulin (IVIg) [68] and IL-15 [89]; only modest benefits, such as a reduction in TB symptoms, *e.g.* IFN-γ [81]; or no overall benefit when larger double-blind randomised trials have been performed, *e.g. Mycobacterium vaccae* [69]. Possible explanations for the wide variation in therapeutic responsiveness include: 1) genetic variations in the nature and magnitude of individual immune responses; 2) incorrect timing of therapy in relation to the course of TB infection given the importance of different immune components at specific time-points *e.g.* Th17 responses may be beneficial early in infection but deleterious late in infection; 3) a lack of adequate understanding of the specific immune response to MDR- and XDR-TB and, hence, the selection of inappropriate therapeutic immune targets; and 4) the failure to deliver therapies to the site of infection [67]. It is unlikely that immunotherapy will be applicable in a "one-size fits all" approach [92]. The timing and nature of immunotherapy will need to be tailored to individuals or particular patient sub-groups based on adequate immune-profiling [67]. For immunotherapy to be successfully applied to DR-TB, accelerated efforts are needed to: better understand the immune response to DR-TB; develop technologies that enable rapid and affordable immune-profiling to guide selection and timing of therapy; and perform phase IIb and III clinical trials of applicable immunotherapies in larger groups of MDR- and XDR-TB patients.

Prognosis of DR-TB

Global outcomes in the treatment of DR-TB vary widely depending on regimen choice, duration of treatment, as well as background prevalence of TB and HIV [53]. While overall treatment success rates of MDR-TB are estimated at ~60%, the use of different regimens leads to significant heterogeneity in the outcome results. The use of individualised treatment regimens resulted in a mean proportion of patients achieving treatment success (completion or cure) of 64% (95% CI 59–68%) [53, 93]. However, treatment success rates as high as 79% have even been recorded using individualised regimens [94]. MDR-TB cases treated with standardised regimens had a lower success rate of 54% (95% CI 43–68%); however, this was not statistically significant [53]. It is likely that longer duration of therapy and directly observed therapy (DOT) could improve outcomes. Indeed, when treatment periods of 18 months were combined with DOT throughout the treatment period, successful treatment outcomes as high as 69% (95% CI 64–73%) were achieved [53]. In high-burden settings such as South Africa, prior to the introduction of HAART, treatment success was as low as 46% with a mortality of 23% [95–97]. This contrasts significantly with other cohorts from Iran and Bangladesh [53, 98]. Of note, HIV-infected patients had mortality twice as high as HIV-uninfected patients and with a significantly higher chance of early mortality [95, 99].

Table 4. Potential immunotherapeutic agents for tuberculosis (TB)

Product/agent	Theoretical mode(s) of action	Outcome(s), reference(s) and comment
Immunomodulatory: "realigning" immune response towards a more favourable one		
High-dose IVIg	Multiple mechanisms not all clear; fully sialylated Fc oligosaccarhides in IVIg preparations downregulate inflammatory genes	Marked therapeutic effect in a murine model when administered early in infection [68] No studies in DR-TB
M. vaccae (heat-killed environmental mycobacterial preparations)	Alters T-cell response towards Th1 profile and directs CD8+ T-cell against epitopes common among mycobacterial strains	No benefits seen in double-blind randomised trials for DS-TB [69, 70] No studies in DR-TB
RUTI (*M. tuberculosis* liposomal preparation)	Used together with initial period of chemotherapy, the idea is to reactivate dormant *M. tuberculosis* and make them more susceptible to killing by vaccine-generated antigen-specific T-cells	In a murine or guinea pig model, it showed some efficacy in controlling TB after initial chemotherapy [71]
16a-bromoepiandrosterone (HE2000)	Unknown mechanism of action	In a murine model of TB, it had therapeutic effect even in absence of chemotherapy [72]
DNA vaccine *e.g.* with HSP65	Heat shock protein (*hsp*) 65 vaccine enhances cytotoxic T-cell activate and decreases Th2 responses	In murine models, it is effective against TB alone and in combination with chemotherapy [73–75]
Dzherelo (plant extracts)	Unknown mechanism of action	Small human studies in MDR (XDR)-TB from the Ukraine show possible benefits [76, 77]
Mesenchymal stromal cells	Downregulation of inflammation-related genes and upregulation of phagocytic genes [78]; tissue regenerative capacity	9-patient study in MDR-TB with favourable outcomes [67]
Immunosuppression to reduce inflammation		
Thalidomide	Suppression of TNF-α, increases IL-2 and IL-12, co-stimulatory effects on T-cells	Beneficial effects demonstrated for severe inflammatory reactions associated with TB [79, 80] No studies in DR-TB
Etanercept	Anti-TNF-α, neutralises TNF-α	Not yet studied as adjunctive TB therapy
Effector cytokine therapy to enhance microbicidal effect		
IFN-γ	Increased Th1 responses, increased IP-10/decreased IL-17 with decreased neutrophil-mediated inflammation [67]	Decrease in constitutional symptoms and increased rate of sputum conversion but this was not sustained and not significant at 2 months [81, 82] Decreased tissue damage improved time to *M. tuberculosis* clearance [83] Small studies in DR-TB [84, 85]

Table 4. Continued.

Product/agent	Theoretical mode(s) of action	Outcome(s), reference(s) and comment
IL-2	Increased Th1 responses	RCT showed slightly prolonged time to sputum conversion in treatment group [86] Aerosolised IL-2 may offer compartmentalised utility [67] No studies in DR-TB
IL-7	Helps to improve immune responsiveness in "immune-exhaustion" states by protection from apoptosis, improving dendritic cell activation and immune memory	Successful use post stem cell transplant and in HIV infection and similar effects may be useful for long-standing TB infection [87, 88] No studies yet in DR-TB
IL-15	Helps to improve immune responsiveness in "immune-exhaustion" states by protection from apoptosis and improvements in immune memory function	In a murine model, showed survival advantage in TB infection if co-administered with BCG [89, 90]
IL-24	Direct activation of CD8+ T-cell in mice, increased IFN-γ production; activates neutrophils and their IL-12 production	In a murine model it had a protective effect against *M. tuberculosis* infection [91]

IVig: intravenous immunoglobulin; DR: drug-resistant; DS: drug susceptible; *M. vaccae*: *Mycobacterium vaccae*; *M. tuberculosis*: *Mycobacterium tuberculosis*; Th: T-helper cell; MDR: multidrug-resistant; XDR: extensively drug-resistant; TNF: tumour necrosis factor; IL: interleukin; IP: inducible protein; RCT: randomised controlled trial; BCG: bacille Calmette–Guérin.; IFN: interferon. Data from [66]. Reproduced and modified from [67], with permission from the publisher.

XDR-TB outcomes are worse than MDR-TB outcomes, with an overall XDR-TB treatment success rate of 44% (95% CI 37.8–54.5%) [3, 93]. Cure rates as high as 60.4% have been documented in some groups from Peru and Korea; however, these cohorts did not include HIV-infected patients [100, 101]. By contrast, the cure rate in high-burden countries is very low, even independent of HIV status, with culture conversion under 20% [3] and mortality as high as 42% [102]. Adverse event profiles in these patients are also high [103]. Nevertheless, even in several low-burden countries, cure rates of XDR-TB are dismal at 29% [101, 104, 105]. Thus, XDR-TB is a marker for poor prognosis.

Ethical and logistical challenges in managing DR-TB in high-incidence countries

Defaulting treatment due to alcohol abuse, drugs and various social factors is a major problem in high-burden settings. Social determinants play a major role in defaulting treatment [106]. One is faced with a problem of what to do with recurrent defaulters where there is longitudinal amplification of drug resistance. We have, on occasion, withdrawn treatment in such patients to prevent further amplification of resistance. In many high-burden settings, there is an inadequate legal framework to deal with such patients. While there is a minority of patients that are unmanageable, violent and pose a threat to healthcare workers, these patients are a reality and are particularly difficult to manage. Incarceration is an option that was used in the control of the MDR epidemic in the US in the 1990s, but is often not enforceable in high-burden settings and appropriate resources are often unavailable. It must be emphasised that these considerations apply to a minority of patients and it is not a strategy that may be successful or enforceable. Access to DR-TB treatment is often unavailable in most countries in Africa, with the exception

of South Africa and a couple of others. Although the MDR-TB treatment programme is being decentralised in South Africa, patients that are ill or patients with XDR-TB still require admission to hospital, and there is often a chronic shortage of hospital beds. This promotes transmission and drives increased morbidity and mortality.

There is also an emerging problem of DR-TB in healthcare workers in high-burden settings [107, 108]. Thus, appropriate infection control measures must be enforced. However, in resource-poor settings, such controls are often lacking or inadequate, thus fuelling transmission to healthcare workers and patients.

Challenges in managing XDR-TB and therapeutically destitute cases of DR-TB

The response rate in patients with XDR-TB in countries like South Africa is often poor with less than 20% of patients responding to medical treatment [3]. While mortality is significant (approximately 40–50%), as in the pre-chemotherapeutic era, approximately 20–25% of patients have chronic ongoing disease. Thus, even in HIV-infected patients, there is an appreciable number of patients who are chronic excreters of bacilli. These patients are therapeutically destitute and do not respond despite the use of appropriate regimens, admission to hospital and injectable agents. This represents a challenging problem and facilities are required to appropriately deal with these patients [3, 13]. Currently, the sheer burden of disease means that they occupy a large proportion of hospital beds in TB facilities.

Thus far, in the Western Cape of South Africa, there have been approximately 100 patients who are therapeutically destitute (failed 12 months on in-patient therapy using appropriate regimens with an injectable agent) and have now been discharged back into the community. A social assessment is performed and a review committee makes the decision on discharge. This poses a serious risk to transmission and amplification of resistance. New approaches are needed and special facilities must be built to deal with such patients in resource-poor settings. This issue has been reviewed in detail recently [13]. In many high-burden countries, including South Africa, the MDR-TB treatment programme has been decentralised. However, XDR patients are still treated within hospitals. Decentralisation of XDR-TB treatment would reduce costs and potentially also the rates of nosocomial transmission. However, peripheral healthcare facilities will first need to be equipped with dealing with treatment monitoring and use of complex drugs such as capreomycin. Management of patients with XDR-TB is complex because of the high rate of adverse drug reactions and the challenges that these patients pose. Input from tertiary care facilities and specialist physicians is therefore often required to adequately manage these cases.

Conclusions

DR-TB poses a serious threat to TB control in high-burden settings. Most of the DR-TB in these settings, including in South Africa and China, is through primary spread while XDR-TB is mainly acquired. However, there is increasing person-to-person spread as the epidemic of XDR-TB progresses and matures. The line probe assays and real-time PCR assays, such as Xpert® MTB/RIF, represent important advances in the diagnosis of drug-resistant TB. However, sub-optimal PPV of tests such as GeneXpert® assay and sub-optimal sensitivity smear-negative TB, remain an unmet research need. Better access is required not only to diagnostic facilities but also to second-line drugs in high-burden settings. Many countries in Africa still do not have treatment programmes for DR-TB. In contrast to intermediate and low-burden settings, the prognosis of DR-TB is poorer in high-burden settings. While strengthening of national TB programmes to prevent the emergence of DR-TB is paramount, steps need to be taken to deal with the existing caseload, and to deal with the growing problem of therapeutically destitute cases of XDR-TB treatment failures, and patients with totally drug-resistant (TDR)-TB. This should remain a major priority for the STOP-TB partnership,

the WHO, international funding agencies, and the TB research community. There is still much work to be done to prevent and manage existing cases of DR-TB in resource-poor settings.

Statement of Interest

R. Dawson has received grant and commercial research funding to conduct EBA studies on: Delamanid, SQ109, PA824, Sutezolid and Bedaquiline.

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Chapter 9

TB drug resistance in low-incidence countries

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SUMMARY: During the last few years, many low-incidence countries have reported a significant decrease in the incidence of tuberculosis (TB) among the local-born population, while the proportion of foreign-born TB patients is increasing. Multidrug-resistant (MDR)-TB is, in general, uncommon in low-incidence countries, although rates are higher in recent entrants, especially among immigrants from countries where prevalence of MDR-TB is high, in previously treated individuals and in HIV-infected patients. Improvements in awareness and management strategies in low-incidence countries are urgently required in order to prevent outbreaks of drug-resistant (DR)-TB in the local populations.

KEYWORDS: Drug resistance, low-incidence countries, tuberculosis

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Countries with a low incidence of tuberculosis (TB) were originally defined in 1991 as those with a crude annual case notification rate <10 per 100,000 inhabitants and declining [1]. Afterwards, the annual case notification rate was extended to define the low-incidence countries. The European framework document on TB control and elimination in low-incidence countries include all countries in Europe with a crude annual notification rate <20 per 100,000 population [2], and in comparison with TB surveillance systems in low-incidence industrialised countries [3], low incidence was defined as <16 new cases per 100,000 population annually during a 4-year period. Furthermore, in the latest World Health Organization (WHO) control report, *Global Tuberculosis Control* 2011 [4], the definition of low-incidence countries is conflicting since it includes countries with an incidence rate of <20 cases per 100,000 population or <10 cases in total as low-incidence ones. Distinctly, in the USA, low-incidence states are defined as areas with annual incidence rates of ⩽3.5 cases per 100,000 population [5]. Due to the lack of a clear definition of a low-incidence country, the original definition from 1991 [1] has been applied in this review.

In the last few years, many low-incidence countries have reported a significant decrease in the incidence of TB among the local-born population, while the proportion of foreign-born TB patients is increasing [6]. For example, in England and Wales (UK), between 2001 and 2003, the TB incidence among immigrants was 22 times higher compared with the UK-born population (88 *versus* 4 per 100,000 population, respectively) [7]. In Denmark, a nationwide 12-year study showed that migrants contributed 61.6% of all detected TB cases [8].

This chapter focuses on the epidemiology and risk factors of drug-resistant (DR)-TB in low-incidence countries, based on the original definition [1]. Concepts of diagnostics and treatment are discussed in more detail in other chapters within this issue of the *European Respiratory Monograph*.

Epidemiology

According to the WHO *Global Tuberculosis Control* report [4], in 2010, there were 52 countries (out of 217) with an annual TB notification rate <10 per 100,000 population (table 1). Of those, 23 were in Europe, 19 belonged to the Americas, six to the Pacific region, three to the Eastern Mediterranean area and one to Africa. In South-East Asia, there were no countries with an annual notification rate ⩽10 per 100,000 population. Of the low-incidence countries, 27 (51.2%) had a population <1,000,000 and 20 (38.5%) had a population <200,000.

The context of TB/HIV co-infection with related challenges is also prevalent in low-incidence countries. In 37 (71.2%) low-incidence countries, at least one TB patient with HIV co-infection has been diagnosed. The highest proportions of TB-HIV co-infected patients were in the Bahamas, Barbados and Jamaica, where 48.4%, 33.3% and 22.3% of all TB patients, respectively, were HIV seropositive. In countries in the European region, the highest proportions of HIV-infected TB patients are found in Malta (15.0%) and the Netherlands (4.6%). In the largest low-incidence country, the USA, 5.5% of all TB patients were HIV seropositive.

The spread of multidrug-resistance (MDR), defined as TB caused by *Mycobacterium tuberculosis* resistant *in vitro* to isoniazid and rifampicin [4], also poses a major challenge to the management of TB in low-incidence countries. Treatment of MDR-TB requires prolonged treatment with second-line anti-TB drugs, which are less effective, more toxic and significantly more expensive than first-line drug-based regimens [9, 10]. As a consequence, the treatment success rates of MDR-TB are substantially lower and the mortality rates notably higher than those of drug-sensitive TB [11]. In the WHO *Global Tuberculosis Control* report, the data on the prevalence of MDR-TB were surprisingly inconsistent in low-incidence countries [4]. Data on MDR-TB were unavailable in 10 low-incidence countries (in nine of those from the European region), whereas data on extensively drug-resistant (XDR)-TB, defined as TB caused by *M. tuberculosis* resistant to isoniazid and rifampicin (*i.e.* MDR-TB), and additionally resistant to any of the anti-TB fluoroquinolones and to at least one of the three injectable anti-TB drugs (capreomycin, kanamycin or amikacin), were not stated. Therefore, the data on the prevalence of DR-TB are reproduced on the basis of the updated report on anti-TB drug resistance surveillance worldwide [12]. The epidemiological data on MDR-TB and XDR-TB in low-incidence countries, where >100 TB cases were detected annually, are provided in table 2. Detailed data from Cuba, Jamaica and the United Arab Emirates were unavailable. Of the remaining 23 countries, 13 (56.5%) had <10 MDR-TB cases. The highest notification rates of MDR-TB were reported in the USA, Italy and Germany with 95 (of which 84.7% were new cases), 67 (50.7% new cases) and 36 (77.8% new cases) MDR-TB cases, respectively. Nine (39.1%) out of the 23 low-incidence countries reported at least one XDR-TB case.

Risk factors for drug-resistance

Predictors of drug resistance are divided into two categories [13]: 1) those facilitating the selection of resistance in the community, such as non-standardised TB treatment and factors associated with the supply or quality of the drugs *etc.*; and 2) immigration and special conditions that appear to make selected population groups more vulnerable to infection with DR-TB. Although factors of both categories can be relevant in any country, this classification roughly refers to the differences between the high-incidence and low-incidence countries, respectively. In particular, factors of the first category mostly contribute to the spread of DR-TB in those countries where the prevalence of MDR-TB has appeared to be high. However, in low-incidence countries, the emergence of DR-TB is related to the second category of factors, which are discussed later in this chapter. In addition,

the transmission of DR-TB, causing cases of primary MDR-/XDR-TB also poses a new challenge in low-incidence countries.

Previous TB treatment

Previous TB treatment has been widely and almost invariably recognised as a predictor of MDR-TB in the majority of the earlier reports originating from different parts of the world [14–17]. In a study by ESPINAL *et al.* [18], the likelihood of MDR-TB increased progressively along with the length of the previous treatment period. Indeed, the longer the treatment, the more likely there are to be deviations from standardised treatment or interruptions, and thus, the higher the probability of generating drug-resistant strains. Today's spread of drug resistance indicates a failure of TB-control efforts due to inadequate case management, interruptions in drug supply, or inadequate drug regimens. The particular role of treatment interruption has been addressed in previous studies, which show that the chance of developing MDR-TB increases among previous TB treatment defaulters [19].

Immigration

According to data from the USA [16, 20, 21] and Europe [7, 17, 22, 23], DR-TB has been significantly associated with immigration. This relationship appeared to be stronger in recent immigrants than among those who had lived in the country for more than 5 years [15]. However, although the highest TB incidence occurred in recent entrants in England and Wales, nearly half of the cases had been UK residents for $\geqslant$5 years [7]. This indicates that the more robust predictor of MDR is probably immigration on its own, rather than the particular status of a recent entrant. In France, two-thirds of MDR-TB patients were born outside the country and being born in Sub-Saharan Africa and in European countries other than France was significantly associated with drug resistance [17]. A study by FALZON *et al.* [24] found that within the European Union, TB patients from the former Soviet Union countries had the highest frequency of both primary and secondary multidrug-resistance. In Germany, 80.4% of all the MDR-TB cases were born in states of the former Soviet Union and among previously treated cases, this proportion was as high as 89.0% [25]. Immigrants from the former Soviet Union were also identified to be at increased risk of MDR-TB in California, USA, between 1994 and 2003 [16]. In the light of results from a recent study from Belarus [26], where the proportions of MDR-TB among new and previously treated TB cases (35.3% and 76.5%, respectively) were the highest ever recorded in the world, it is important to intensify immigrant screening from countries with a high prevalence of MDR-TB. The alarming situation in the former Soviet Union countries may stem from an inadequate public health approach to TB control lasting two decades and, particularly, to stock-outs of first- and second-line drugs leading to virtual monotherapy, especially during the collapse of the Soviet Union, as well as the use of potentially substandard-quality drugs and inadequate clinical practices [27].

Furthermore, TB among the foreign-born population in low-incidence countries characteristically tends to affect the younger age cohorts. In a study from the Czech Republic by BARTU *et al.* [28], MDR-TB patients born in the country were 15 years older than immigrants (mean age 48 *versus* 33 years, respectively). In England and Wales, non-UK-born TB patients were 11 years younger than UK-born patients (median age 33 and 44 years, respectively).

HIV infection

There is a strong, unambiguous and well-documented association between HIV and TB. People living with HIV, who are also infected with *M. tuberculosis*, are at 21–34 times greater risk of developing clinical TB compared with those who are HIV negative [4]. Recent extensive investigations have revealed an association between MDR-TB and HIV infection, which has surprisingly appeared to be characteristic of countries of low TB incidence. In particular, most studies from North America and Western Europe [17, 29] have demonstrated a positive association between HIV infection and MDR-TB, which contrasts with studies from Africa, where not a single

Table 1. Tuberculosis (TB) epidemiological data in low-incidence countries in 2010

Country	Notification rate n	All TB cases n	Positive treatment outcome# %		HIV-positive cases n	MDR-TB cases n	Proportion of MDR-TB cases %	
			New smear-positive cases	Re-treatment cases			New cases	Previously treated
African region								
Mauritius	9	122	88	60	8	2	0.9	16.7
Region of the Americas								
Anguilla	7	1			0	0	0	0
Antigua and Barbuda	7	6	67	100	5	0	0	0
Aruba	6	6						
Bahamas	9	31	71	80	15	0	0	0
Barbados	2	6	100		2	0	0	0
Bermuda	2	1	0		0	0	0	0
British Virgin Islands	4	1	100		0	0	0	0
Canada	4	1322	75	64	23	15	1.2	0
Cayman Islands	7	4	50		0	0	0	0
Cuba	7	827	90	74	53	7	0.3	8.9
Curacao	3	5			0	0	0	0
Grenada	4	4	50		1	0	0	0
Jamaica	5	130	69	74	29	1	0	5.3
Montserrat	0	0			0	0	0	0
Puerto Rico	2	80	81		14	0	0	0
Saint Kitts and Nevis	4	2	80		0	0	0	0
Saint Lucia	5	9	57	33	0	0	0	0
Saint Maarten	8	3			0	0	0	0
USA	4	11181	60		612	92	0.7	
Eastern Mediterranean region								
Jordan	5	338	92	80	0	10	1.5	16.7
United Arab Emirates	2	131	73	80	2	0	0	0
West Bank and Gaza Strip	<1	31	82		0	0	0	0
European region								
Andorra	8	7	100	100	0	0	0	0

Table 1. Continued.

Country	Notification rate n	All TB cases n	Positive treatment outcome# %		HIV-positive cases n	MDR-TB cases n	Proportion of MDR-TB cases %	
			New smear-positive cases	Re-treatment cases			New cases	Previously treated
Austria	4	358	67	41		15	1.4	10.3
Belgium	8	810						
Cyprus	4	44						
Czech Republic	6	641						
Denmark	5	295			0			
Finland	6	312	67	36	4	6	1.6	6.7
France	5	2906						
Germany	4	3436	69	59		43	0.8	2.8
Greece	4	466						
Iceland	7	22	75	100		0	0	0
Ireland	7	319	66	60	12	2	0.6	
Israel	5	340	86	67	13	12	3.5	
Italy	3	1721						
Luxembourg	5	24				0	0	0
Malta	5	20	80	50	3	1	0	33.3
Monaco	3	1						
The Netherlands	6	1029	80	72	47	11	1.0	2.3
Norway	5	261						
Slovakia	7	386	82	82	1	1	0	1.8
Slovenia	8	169	87	88	1	0	0	0
Sweden	6	552	85	69		18	2.0	13.5
Switzerland	4	323				9	0.3	7.3
Western Pacific region								
American Samoa	6	4	100		0	0	0	0
Australia	5	1187	79	66	28	33	1.8	16.7
Cook Islands	0	0			0	0	0	0
New Zealand	7	301	76	67	2	4		
Samoa	8	14	90		0	0	0	0
Tokelau	0	0			0	0	0	0

MDR: multidrug-resistant. #: cured or completed. The data are from 2009. Data from [4].

Table 2. Anti-tuberculosis (TB) drug resistance surveillance data in low-incidence countries with >100 registered TB patients annually, 2007–2010[#].

Country	Year	New cases		Previously treated cases		XDR-TB cases	
		Cases with DST results n	MDR-TB %	Cases with DST results n	MDR-TB %	Year	Proportion of MDR-TB cases n (%)
Australia	2010	868	2.4	48	22.9	2010	1 (3.1)
Austria	2010	240	2.1	16	18.8	2010	1 (6.7)
Belgium	2009	621	0.6	56	5.4	2009	3 (30.0)
Canada	2010	987	1.5	51	0.0	2010	1 (7.1)
Czech Republic	2009	413	1.2	39	7.7	2008	1 (10.0)
Denmark	2009	209	0.5	33	3.0	2007	0 (0.0)
Finland	2010	239	2.1	3	12.5		
France	2009	1304	1.0	106	13.2		
Germany	2010	2138	1.3	130	6.2		
Greece	2009	140	6.4	14	28.6	2009	3 (33.3)
Ireland	2010	176	1.1	21	0.0		
Israel	2010	245	4.9	2	0.0	2010	1 (8.3)
Italy	2009	1051	3.2	264	12.5	2009	1 (3.1)
Jordan	2009	95	6.3	7	28.6		
Mauritius	2010	105	1.0	7	14.3		
The Netherlands	2010	741	1.3	29	3.4		
New Zealand	2009	237	2.5	8	12.5		
Norway	2009	210	3.8	20	0.0	2008	0 (0.0)
Slovakia	2010	185	0.0	32	3.1	2010	0 (0.0)
Slovenia	2009	167	0.5	8	0.0		
Sweden	2010	440	2.5	30	23.3	2009	0 (0.0)
Switzerland	2010	270	0.4	33	9.1	2010	0 (0.0)
USA	2010	6514	1.1	293	4.4	2010	1 (1.7)

XDR: extensively drug-resistant; DST: drug-susceptibility testing; MDR: multidrug-resistant. [#]: data unavailable for Cuba, Jamaica, United Arab Emirates, and the West Bank and Gaza Strip. Reproduced and modified from [12] with permission from the publisher.

study demonstrated such a relationship [30]. The results of numerous studies indicate that primary but not acquired MDR-TB is associated with HIV infection [31, 32].

There are multiple reasons why DR-TB is linked to HIV. The first one is acquisition of rifampicin resistance by HIV-infected patients during treatment for TB. Malabsorption of certain anti-TB drugs, especially that of rifampicin and ethambutol, has been documented in settings where HIV prevalence is high [33]. This suggests that HIV-positive TB patients may be at greater risk of acquiring resistance due to the decreased bioavailability of the drugs in question, which equates to the effect of interrupted or non-standardised therapy in terms of the performance of the drugs. Secondly, reasons related to the so-called common exposures may play a role. HIV-positive patients and DR-TB patients may share similar risk factors; for example, history of hospitalisation, intravenous drug abuse, previous imprisonment, socioeconomic distress, or alcohol abuse [30, 33, 34]. Thirdly, an observed association could be set up by time window. HIV-negative patients are likely to reactivate a latent TB infection (LTBI) acquired decades earlier, whereas HIV-infected patients are likely to reactivate a TB infection, acquired more recently from the community or by institutional transmission, to a rapidly progressing disease [30]. Although an association between HIV infection and MDR-TB has been widely documented in hospital outbreaks of DR-TB among people living with HIV [34], current population-based data suggest that the relationship between MDR-TB and HIV is not consistent across all settings.

In the latest surveillance report on DR-TB [12], drug resistance data from 17 countries were combined and stratified by HIV status, but no association was found between the presence of MDR-TB and HIV status. However, the unavailability of data on HIV serostatus from many countries dictates caution in interpreting this lack of association, as in 2011, only 34% of notified TB cases worldwide were tested for HIV [4]. For instance, in a German study, only 80% of the MDR-TB cases and 57% of the XDR-TB cases were tested [25]. Therefore, HIV-testing should be reinforced in TB patients.

Age and sex

An association between MDR-TB and age <65 years has been pointed out in Europe [22]. In a study from Spain [35], an association between MDR and 45–64-years age group was found, whereas in South Korea, MDR-TB was significantly linked to age <45 years [36]. According to the 2010 global report on surveillance of MDR/XDR-TB [37], where data from 27 countries were analysed, in the high-income/low-incidence countries, the frequency of MDR-TB declined linearly along with decreasing age, but in the countries of Central and Eastern Europe, it just peaked at young adulthood.

The issue of sex in association with developing MDR-TB is also intriguing. It has been demonstrated that MDR-TB patients in Western Europe are more likely male [22]. A hypothesis exists that females are more compliant with treatment and therefore less likely to receive inadequate or intermittent treatment. In contrast, in countries of the former Soviet Union and central Asia, where the risk of transmission of DR-TB is greater because of wider spread of the MDR-TB infection, female sex was found to be a predictor of MDR-TB [38–42].

Nevertheless, Zignol *et al.* [12] disaggregated drug-resistance data from 58 countries by sex and noted no association between the presence of MDR-TB and the sex of the patient. Thus, in countries where an association between sex and drug resistance is documented, possible additional co-factors should be carefully considered.

Risk-taking behaviours and lifestyle

MDR-TB has been found to be associated with socially disadvantaged patients, such as the homeless, unemployed [19], intravenous drug users [14] and alcohol abusers [35]. Several reports have shown that socially disadvantaged patients, such as alcohol abusers and homeless people, are at increased risk of defaulting from treatment and treatment failure, and thus have increased risk

for developing drug resistance [43]. In prior reports, MDR-TB cases were much more likely to have a smear-positive cavitary pulmonary disease when compared with non-MDR-TB patients [16]. This phenomenon is most likely related to patient-related diagnostic and treatment delay, which can contribute to the spread of drug-resistant strains. In one study, "known TB contact" and "healthcare worker employment" have been shown to be independent predictors of MDR [14].

Overcrowding

Outbreaks of MDR-TB are reported in settings with a high prevalence for the condition. In such settings cases can be health workers in clinics, intravenous drug abusers, prisoners, *etc.* [44, 45]. Overcrowding in prisons and the inability to isolate resistant cases due to the lack of isolation facilities, clearly increase the transmission of resistant *M. tuberculosis* strains. This fact is internationally well documented and an association of MDR-TB either with being a prisoner or with having a history of previous incarceration has been observed in numerous studies [19, 22].

Other risk factors

Recently, the pharmacokinetic variability between patients has also been suggested as an important risk factor for the occurrence of drug resistance [46]. Additionally, insufficient infection control in hospital settings during the management of an MDR/XDR case can be another critical point that is not necessarily only attributable to high-incidence countries. A recent survey performed in European low-incidence countries showed a significant non-compliance with infection-control recommendations [47], although hard evidence on nosocomial transmission of TB is scarce.

XDR-TB risk factors

In contrast with MDR-TB data, there is little, if any, research information available on the predictors of XDR-TB. The risk factors associated with XDR-TB in low-TB incidence countries are analysed only in two studies with very low patient numbers, one from the USA [48] and another from Germany [25], where 83 and seven XDR-TB cases were analysed, respectively. In a descriptive analysis from the USA, where all TB cases reported from 1993 to 2007 were included, patients with XDR-TB, compared with those with MDR-TB, were more likely to have disseminated TB, were less likely to convert to a negative sputum culture and were infectious for a longer time (median time to culture conversion 183 days in XDR-TB patients *versus* 93 days in MDR-non-XDR-TB patients).

Other studies on XDR-TB risk factors have been performed in intermediate- and high-incidence countries with an overall high prevalence of DR-TB. In the first published studies on XDR-TB risk factors from South Korea, the presence of bilateral cavities at the time of the diagnosis of MDR-TB [49] and the cumulative duration of previous treatment of 18–34 months were significantly associated with XDR-TB [50]. In an analysis that included all XDR-TB cases diagnosed in a Portuguese hospital between April 1999 and June 2007 (n=69), TB/HIV co-infection and increased duration of previous treatments were significant predictors of XDR-TB [51]. In a cross-sectional study from Estonia, which included all XDR-TB patients notified from 2005 to 2007 (n=60), XDR-TB was associated with previous anti-TB treatment, HIV-infection, homelessness and alcohol abuse [52].

Diagnosis of DR-TB

The diagnosis of DR-TB should follow state-of-the-art concepts and involve molecular TB diagnostics, molecular resistance testing and rapid culture methods [53]. The use of molecular methods for testing resistance against first- and second-line drugs needs to be promoted in the light of the assumption that a rapid and adequate initiation of correct treatment is consistently associated with better treatment outcome and reduced transmission rates [54].

Treatment of DR-TB

In low-incidence settings, treatment of MDR/XDR-TB should follow the recent WHO guidelines, which are based on the best available evidence [55, 56]. Since 2011, the new compound TMC 207 (Bedaquiline) has become available in several European countries, through a compassionate use programme for treatment of selected, highly resistant cases, representing an important and encouraging extension of the treatment portfolio.

Treatment outcomes of DR-TB

MDR-TB should be considered a treatable disease, but the management of MDR-TB requires use of second-line drugs, which are less effective, more toxic and more expensive than the first-line drug-based regimens [9, 10]. As a consequence, the treatment success rates in MDR-TB cases are substantially lower than those of drug-sensitive TB cases [11, 57–60]. In a meta-analysis, ORENSTEIN *et al.* [61] showed successful treatment outcomes in 64% of patients treated according to individualised regimens. XDR-TB has shown even lower rates of successful treatment outcomes [49, 62, 63], especially when aggravated by a concomitant HIV infection [64]. A study from Italy and Germany demonstrated for the first time in Europe that HIV-negative XDR-TB patients have a five-fold higher risk of death than MDR-TB cases and need longer treatment, longer hospitalisation and a longer time to convert their sputum smear and culture [62]. Similar results were reported in a large cohort study from four European countries with low HIV prevalence [65]. In that study, XDR-TB cases had a worse clinical outcome than MDR-TB cases resistant to all first-line anti-TB drugs; susceptibility to one or more first-line drug increased the probability of successfully treating MDR-TB cases. With the currently available drugs, XDR-TB patients are left with few, if any, treatment options. In the first systematic review available on XDR-TB, comparative analysis confirmed that those patients have, in general, a poorer prognosis and fewer treatment options than MDR-TB cases [66]. Data show that XDR-TB can be successfully treated in up to 60% of patients, particularly those who are not co-infected with HIV [67]. However, treatment duration is longer and outcomes are in general poorer than for non-XDR-TB patients. A national survey in Germany, representing 37% of all culture-confirmed TB cases in the 3-year period, showed that in conditions of extensive drug-susceptibility testing (DST) and availability of second- and third-line drugs under in-patient management relatively high treatment success rates, 59.3% for MDR-TB patients and 57.1% for patients with XDR-TB (n=7), were reported [25].

The first meta-analysis on XDR treatment outcomes included 13 studies, 10 of which were performed in low-incidence countries. The proportion of favourable outcomes was only 44% [68].

Other studies suggest that the management of XDR-TB can be successful within the existing treatment strategies for MDR–TB, by using aggressive medical and, if needed, surgical treatments, and involving enormous financial investments [66, 69–71]. Taking this into account, the growing number of MDR/XDR treatment failures poses a huge problem, particularly for low-income countries, which to date has not been sufficiently addressed [72].

Programmatic challenges of MDR/XDR-TB management in low-incidence countries

A recent survey of national clinical reference centres in five European countries with different epidemiological patterns identified relevant mistakes and deviations from the recommended practices in the management of TB and MDR/XDR-TB cases in Europe [73]. Deviations from the international standards of TB care were observed in the following areas: surveillance (no information available on patient outcomes); infection control (lack of respiratory isolation rooms/procedures and negative-pressure ventilation rooms); clinical management of TB, MDR-TB and HIV co-infection (inadequate bacteriological diagnosis, regimen selection and treatment duration);

laboratory support and diagnostic/treatment algorithms. The shortcomings identified and recent new epidemiological data require implementation of targeted measures, as addressed in the recent *Roadmap to prevent and combat drug-resistant tuberculosis*, issued by WHO Europe [74].

The management of MDR/XDR cases in low-incidence countries is characterised by various challenges. In the authors' view, the following points are particularly relevant for low-incidence countries. New screening strategies of recent migrants and high-risk groups might contribute to the control of MDR/XDR-TB in low-incidence settings, although the most cost-effective strategy has not yet been defined and the enhanced disease control efforts in the countries of origin are still the main goal [75]. The lack of national treatment programmes, which is the case in most Western European countries, complicates the coordination and administration of MDR/XDR treatment and the adequate monitoring of such patients. With regard to the long and complicated therapy, national or even international consultation services, combined with dedicated interventions by the public health authorities, are required to warrant better treatment completion and success rates [76]. Models of care for failures of MDR/XDR-TB treatment need to be established. While the management of TB contacts has a strong base of evidence, the best ways to manage MDR/XDR-TB contacts are, in contrast, unknown and create many uncertainties. Trials designed to answer these questions are urgently needed [75]. Finally, awareness and knowledge about MDR/XDR-TB among physicians in general is low. Continued medical education and information, particularly about risk factors of drug resistance and new molecular diagnostics, need to be improved [77].

Conclusion

In low-incidence countries, the overall rate of TB is low, though it is far from being eliminated. The majority of cases occur in recent immigrants, and MDR-TB is generally uncommon. MDR-TB rates are higher in recent entrants, especially among: 1) migrants from countries in which prevalence of MDR-TB is high; 2) previously treated individuals; and 3) HIV-infected patients. In low-incidence countries, improved awareness of DR-TB and the consequent application of evidence-based management strategies is urgently required.

Statement of Interest

None declared.

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Chapter 10

Diagnosis of TB: state of the art

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SUMMARY: Rapid, affordable and accurate tuberculosis (TB) diagnosis is key to effective patient management and global TB control. Effective clinical screening and optimised sample acquisition methods remain the first steps in the diagnostic process. Smear microscopy, despite optimisation, remains widely used even though its sensitivity is poor. Mycobacterial liquid culture is accurate but poorly accessible. The use of novel molecular tools, such as Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) or GenoType® MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) assays, which offer superior diagnostic accuracy and decreased time-to-diagnosis for drug-sensitive and/or -resistant TB, is increasing following World Health Organization (WHO) endorsement and, in some countries, national roll-out is underway. In contrast, both serology (antibody-detection tests) and interferon-γ release assays (IGRAs) have been found to offer little diagnostic utility for active TB diagnosis and have been discouraged by WHO. IGRAs and the tuberculin skin test (TST) remain important tools for latent TB infection (LTBI) diagnosis. Other novel, simple technologies, such as the point-of-care (POC) urine lipoarabinomannan strip test and the visually read loop isothermal amplification PCR nucleic acid amplification technique (NAAT), although of uncertain and restricted clinical utility, highlight the progression toward an inexpensive, instrument-free, laboratory-free POC diagnostic technology for TB in the future.

KEYWORDS: Diagnosis, tuberculosis

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Affordable, accurate and rapid diagnosis followed by effective therapy is the cornerstone of tuberculosis (TB) control. TB is curable in over 95% of cases but the same diagnostic standard remains elusive to many who need it most. Multiple social, host and pathogen factors, as depicted in figure 1, intersect to produce and worsen TB diagnostic delay or failure. Fortunately, thanks to renewed global awareness, financial investment and international collaboration, several new diagnostic options have been developed. Old tools continue to be optimised and several new tools are now commercially available and being scaled up by national TB programmes.

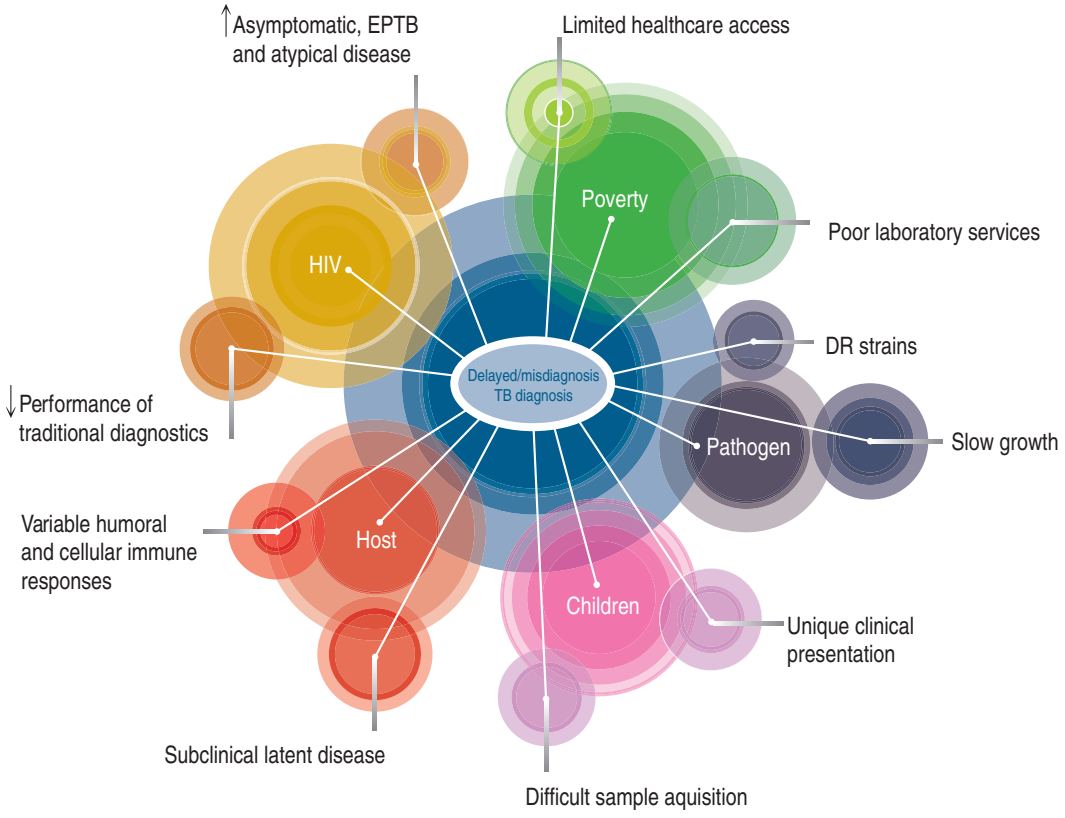


Figure 1. The multiple inter-related factors driving misdiagnosis or delayed diagnosis of tuberculosis (TB). The diameters of the larger circles indicate the relative impacts on delayed/misdiagnosis of TB and the overlapping circles indicate relatedness. EPTB: extrapulmonary tuberculosis; DR: drug-resistant.

This chapter will discuss the optimisation of old tools, the development and integration of new diagnostic technologies for active TB (including smear-negative TB), and drug-susceptibility testing (DST), extrapulmonary TB (EPTB) and latent TB infection (LTBI). It also aims to highlight the progress towards novel point-of-care (POC) TB diagnostics. In particular, diagnostic test performance characteristics and optimal settings for use will be discussed, finally emphasising research "gaps" and the ongoing unmet diagnostic needs.

Diagnosis of active pulmonary TB and DST

Table 1 describes current, commercially available TB diagnostics for active TB and DST stratified by the test's ability to provide diagnosis and DST alone or combined. In addition, to better contextualise available old and new tests, figure 2 visually contrasts the test performance and time-to-result of commercially available diagnostics for active TB and DST.

Improving the old

Clinical case definitions and symptom screening

Clinical screening, diagnosis and case definitions continue to guide treatment decisions for active TB, and form part of the composite diagnostic reference standards. In recent years, researchers have begun to objectively evaluate the performance of some of the available clinical and radiology-based diagnostic guidelines, reach consensus on clinical case definitions for certain TB forms with suboptimal reference standards (*e.g.* TB meningitis) and use screening to strengthen World Health Organization (WHO) guidelines.

Table 1. Commercially available diagnostics for active tuberculosis (TB) and drug-susceptibility testing (DST)

Test type or platform	Description of test	Current validated commercial versions	Low/high sensitivity (expected)	Low/high specificity (expected)	WHO endorsement	Comments
Detection of active TB						
Fluorescent microscopy using LED	Auramine O-stained smear read by fluorescent microscopy using LED light source	Primo Star iLED™ (Carl Zeiss, Oberkochen, Germany) Lumin™ (LW Scientific, Lawrenceville, GA, USA) and others	56–80% (concentrated, direct samples compared with culture) [1–4]	92–98% (compared with culture) [2–4]	Yes	Approximately 10% greater sensitivity *versus* ZN light microscopy No reduction in performance in HIV co-infection [1] WHO endorsed
Semiautomated, nonintegrated NAAT	Amplification and detection of mycobacterial rRNA or DNA direct from clinical samples	Amplified MTD® (GenProbe, San Diego, CA, USA) Probe Tec ET (BD, Franklin Lakes, NJ, USA) Cobas Taqman MTB (Roche Molecular Diagnostics, Pleasanton, CA, USA)	36–100% (pooled approximately 66–96%) [5–7]	54–100% (pooled 85–98%) [5–7]	No	Sensitivity in smear-negative patients: 50–80% [6, 7] Open system at risk of DNA contamination and specificity affected by laboratory quality control
Simplified, manual NAAT	Isothermal amplification with visual readout to detect mycobacterial DNA direct from clinical samples	Eiken LAMP® (Eiken, Tokyo, Japan)	Overall: 83%; smear-positive patients: >95%; smear-negative: 41–52% [8, 9]	>97% [8]	No	This test is undergoing large-scale evaluation and demonstration studies by FIND so should not be considered fully validated; evidence to be reviewed by WHO expert group in early 2012
Serological (antibody) detection test	Immunological test: detection of antibodies to TB antigens by ELISA or rapid lateral flow format	Although several assays are on the market, no currently available test has been validated and proven to be clinically useful	0–100% [10] Anda-TB (Anda Biologicals, Strasbourg, France) IgG: pooled estimates in smear-positive patients, 76%; smear-negative, 59%	31–100% [10]; Anda-TB IgG pooled estimate: 92%	No	WHO made negative recommendation in 2011
Antigen detection test	TB antigens detected by ELISA or lateral flow test format	TB LAM ELISA (Alere, Waltham, MA, USA)	Overall: 18–59%; HIV only: 20–67% [11]	88–100% [11]	No	Only offers clinical utility in HIV-infected patients with advanced immunosuppression Validation of lateral flow strip test (Determine TB®; Alere) ongoing
Detection of active TB and DST						
Fully automated, integrated NAAT	Fully automated, self-contained platform integrating sputum processing, mycobacterial DNA extraction and amplification	Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA)	*M. tuberculosis* detection: overall, 90%; smear-positive patients, 94–100%; smear-negative patients, 46–83% (pooled sensitivity 75%); rifampicin resistance, 98–99% [12–15]	*M. tuberculosis* detection: >98%; rifampicin resistance: >98% [12]	Yes	WHO strong recommendation for frontline TB diagnosis in HIV-infected and MDR-TB suspects
Automated liquid culture with indirect DST	Automated system for mycobacterial liquid culture and subsequent DST	Bactec™ MGIT™ 960 (BD Diagnostics, Sparks, MD, USA) BacT/ALERT® 3D (bioMerieux, Marcy l'Etoile, France)	Smear-positive patients: 100%; smear-negative patients: >75%	>99%	Yes	Approximately 10% higher diagnostic yields compared with solid-media culture Contamination can be 10–20% in laboratories with poor quality assurance Indirect DST can take 3–4 months to provide results
Nonautomated liquid culture with direct DST	Simplified systems for mycobacterial liquid culture with reduced laboratory equipment for MTB detection and direct DST	TB MODS Kit® (Hardy Diagnostics, Santa Maria, CA, USA)	96% (compared with traditional automated liquid culture) [16]	96% (compared with traditional automated liquid culture) [16]	Yes	Low equipment requirements offset by high labour needs Direct DST provides results in 10–14 days

Table 1. Continued.

Test type or platform	Description of test	Current validated commercial versions	Low/high sensitivity (expected)	Low/high specificity (expected)	WHO endorsement	Comments
Phage-based detection	Bacteriophage viruses infect and detect the presence of viable *M. tuberculosis*	FASTPlaque™ (Biotec Laboratories Ltd, Ipswich, UK)	81–100% [17, 18]	73–100% [17]	No	3–36% indeterminate rate limits use
DST and/or speciation						
Manual amplification and hybridisation (LPA)	NAAT with hybridisation of amplified product to strip test allowing for identification of *M. tuberculosis* and common mutations causing resistance to rifampicin and isoniazid	GenoType® MTBDR*plus* (Hain Lifescience GmbH, Nehren, Germany) INNO®-LiPA Rif.TB (Innogenetics, Ghent, Belgium)	For rifampicin resistance: >98%; for isoniazid: >84% [19, 20]	For rifampicin resistance: >98%; for isoniazid: >99%	Yes	Provides DST results on culture isolates and smear-positive clinical specimens in 1–2 days Undergoing widespread scale-up in NTPs with the help of the EXPAND-TB programme
Rapid speciation assay	Rapid immunochromatographic (lateral flow) test for identification of *M. tuberculosis* complex in culture isolates	Capilla TB-Neo® (Tauns, Tokyo, Japan) TBc ID® (BD Diagnostics) SD Bioline Ag MPT64 Rapid® (Standard Diagnostics Inc., Yongin, South Korea)	>98%	>99%	Yes	Simple, reliable tests for use especially in settings with high rates of NTMs

WHO: World Health Organization; LED: light-emitting diode; NAAT: nucleic acid amplification technique; LPA: line probe assay; rRNA: ribosomal RNA; *M. tuberculosis*: *Mycobacterium tuberculosis*; Ig: immunoglobulin; ZN: Ziehl–Neelsen; FIND: Foundation for Innovative New Diagnostics; MDR: multidrug-resistant; NTP: national TB programme; EXPAND-TB: Expanding Access to New Diagnostics for TB; NTM: non-tuberculous mycobacterium.

WHO recently used the findings of a large meta-analysis of symptom screening to inform the intensified case finding and isoniazid preventive therapy guideline for persons living with HIV [21]. This study found that, at TB prevalence rates of 5 and 20%, the absence of current cough, fever, night sweats or weight loss reliably excluded active TB in 98% and 90% of patients, respectively [22]. The 2006 WHO smear-negative TB diagnostic algorithm was recently evaluated in ambulatory patients attending an outpatient clinic, and was found to have a sensitivity and specificity of only 80% and 44%, respectively [23]. However, despite this modest diagnostic accuracy, another study in hospitalised patients suggested that the strict use of these clinical guidelines could reduce 8-week mortality and hospital length of stay [24]. Finally, concerted efforts are underway to unify clinicoradiological case definitions for different forms of TB (*e.g.* TB meningitis) to allow for better comparative assessment across studies and evaluate diagnostic performance more consistently across settings [25].

Chest radiology

Radiology is widely used in both high- and low-burden settings for both TB screening in asymptomatic patients and the diagnosis of active disease [26]. Used alone, chest radiology has only moderate specificity and, in settings of high HIV prevalence, moderate sensitivity [27], with 10–71% of HIV co-infected TB patients having an entirely normal chest radiogram despite culture-positive disease [28–31]. However, when chest radiology is used in conjunction with other simple diagnostic tools, such as symptom screening and/or smear microscopy, it can offer both diagnostic utility and cost-efficacy, particularly for ruling out active TB disease [32–34]. Two South African studies found that the combination of symptoms with or without sputum smear microscopy followed by chest radiology offered a negative predictive value of more than 95% for active TB in a high-burden setting [35–37]. Unfortunately, optimal chest radiology utility requires interpretation by trained, skilled observers, which are not always available. Interobserver variability of chest radiology has been shown to be poor, irrespective of reader skill [27, 38, 39]. To overcome this drawback, several radiological

scoring systems, such as the Chest Radiograph Reading System, have been developed to improve interobserver variability [31]. Furthermore, automated computer systems to interpret and report digital chest radiograms are currently in development [40].

Sample acquisition technology

Definitive TB diagnosis relies on the demonstration of TB organisms, or TB-specific antigens or genetic material. For this, an appropriate and sufficient biological sample is essential. Attaining adequate samples can, however, be challenging and a major obstacle to diagnosis. A number of strategies and techniques have been evaluated to improve sputum expectoration, to induce sputum or to attain an alternative pulmonary sample suitable for laboratory TB diagnosis (table 2).

Of these techniques, sputum induction is emerging as the optimal technique given its safety, efficacy and feasibility even in resource-limited settings. The challenge lies in successfully integrating sputum induction into busy, routine clinical practice settings with limited resources.

Smear microscopy

Although widely used, the sensitivity of smear microscopy is highly variable, ranging between 20% and 80% [62], performing poorest in HIV-infected patients [27] and children [63]. Additionally, smear microscopy relies on well-trained microscopists, and sensitivities between field and reference laboratories can vary by as much as 28% [14].

The most important developments in optimising smear microscopy and associated WHO policy changes are outlined in figure 3a. In addition, several innovative approaches to further improve smear microscopy are under development, including improved concentration techniques using nanobeads, fluorescence *in situ* hybridisation, automated and computer-assisted smear reading technologies, and use of mobile phones for microscopy [64, 65].

The expansion and development of culture-based techniques

Mycobacterium tuberculosis culture remains the clinical and research diagnostic gold standard for all forms of active TB. Figure 3b outlines the progress and associated WHO policy changes for mycobacterial culture techniques for both diagnosis and DST. Traditional solid culture methods are tedious, time-consuming and have limited clinical impact. Automated liquid culture systems, with approximately 10% higher yields and a decreased time to diagnosis [66], have largely replaced solid culture. However, automated liquid cultures are expensive, prone to contamination, and require considerable laboratory infrastructure and expertise. Thus, despite WHO endorsement in 2007, they remain inaccessible to populations where they are most needed. In 2009, the WHO endorsed the use of alternative, simpler, less expensive noncommercial culture and DST technologies, as an interim measure while the capacity for genotypic testing is scaled up [67]. Details of the microscopy observed drug susceptibility (MODS) method are shown in table 1, while other endorsed noncommercial methods include the colorimetric redox indicator and the nitrate reductase assay. Under controlled laboratory conditions, these noncommercial culture methods are inexpensive and can provide culture and DST results in 7–14 days [67, 68]. Lack of standardisation and local variations in methodology remain programmatic concerns and have thus far limited scale-up. Phage-based methods have not been WHO-endorsed due to insufficient evidence, variable specificity and high rates of invalid results [69].

Ushering in and tailoring the new

Nonintegrated, semiautomated nucleic acid amplification techniques

Conventional, nonintegrated nucleic acid amplification techniques (NAATs) (table 1) have been found to offer high specificity (85–98%) and sensitivity for smear-positive TB (~96%), but poorer

sensitivity (~60%) and specificity for smear-negative TB [5–7]. Compared with smears and culture, these assays are expensive, requiring specialised laboratory infrastructure and expertise, while, being open systems, they are at risk for cross-contamination in settings with suboptimal laboratory quality. These factors have limited their widespread uptake in high-burden, resource-limited settings. Simplified, manual NAATs, such as LAMP® (loop isothermal amplification PCR; Eiken, Tokyo, Japan) using isothermal amplification and a visual readout have been developed as more affordable options where laboratory infrastructure is limited [8]. Early evaluation suggested similar performance to other commercial NAATs with a sensitivity of ~40% in smear-negative TB [8]. The Foundation for Innovative New Diagnostics (FIND) is currently conducting large-scale evaluation and demonstration studies of LAMP, with promising preliminary results [9]. However, despite the assay's simplicity, the risk of cross-contamination during manual DNA extraction and need for laboratory training and technical skill remain, and may prevent widespread application.

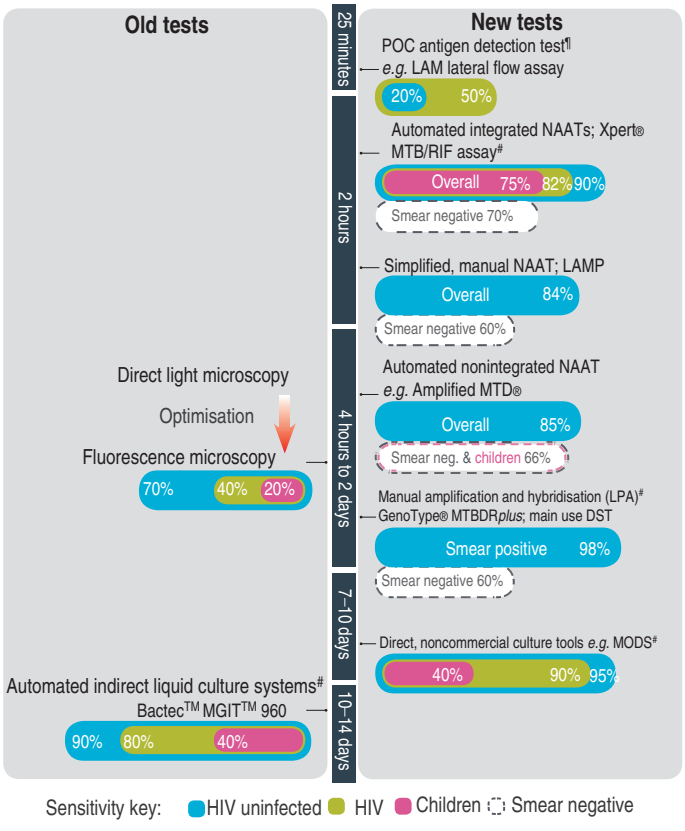


Figure 2. Comparison of the sensitivity and time to diagnosis for active pulmonary tuberculosis (TB) and drug-susceptibility diagnostic tools indicating areas of reduced performance in children and HIV-TB co-infected patients. Only tests commercially available and with a specificity of >95% for the diagnosis of active TB are included. Bactec™ MGIT™ 960 is manufactured by BD Diagnostics (Sparks, MD, USA). Xpert® MTB/RIF is manufactured by Cepheid (Sunnyvale, CA, USA). Amplified MTD® is manufactured by GenProbe (San Diego, CA, USA). GenoType® MTBDR*plus* is manufactured by Hain Lifescience GmbH (Nehren, Germany). POC: point-of-care; LAM: lipoarabinomannan; NAAT: nucleic acid amplification technique; LAMP: loop isothermal amplification PCR; LPA: line probe assay; DST: drug-susceptibility testing; MODS: microscopy observed drug susceptibility. #: diagnostic test that can be used for both *Mycobacterium tuberculosis* detection and DST; ¶: LAM lateral flow strip test is restricted to use in HIV-infected patients and will only be commercially available in the fourth quarter of 2012.

An integrated, fully automated NAAT: Xpert® MTB/RIF

In December 2010, the WHO announced the endorsement of the novel Xpert® MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) [70]. Xpert® MTB/RIF is a fully automated and integrated DNA extraction and amplification system, thereby addressing many limitations of existing commercial NAATs [71]. Furthermore, Xpert® MTB/RIF has the potential to be performed in decentralised locations outside of reference laboratories by staff with minimal laboratory training (1–2 days).

To date, the Xpert® MTB/RIF assay has undergone evaluation in sputum samples from more than 11,000 patients in 19 countries [12–15, 61, 72–74], although these studies have performed Xpert® MTB/RIF in a laboratory, rather than at the POC. A meta-analysis of these 18 published studies showed a sensitivity and specificity of a single sputum-based Xpert® MTB/RIF for culture-positive TB of 90.4% (95% CI 89.2–91.4%) and 98.4% (95% CI 98.0–98.7%), respectively, and 75% for smear-negative, culture-positive pulmonary TB [12]. Performing a second and third MTB/RIF increases sensitivity by approximately 13% and 5%, respectively [15], while indeterminate rates of

Table 2. Strategies/techniques for the improved acquisition of pulmonary samples for tuberculosis (TB) diagnosis

Technique	Diagnostic performance ranges[#]	Advantages	Disadvantages	Knowledge gaps
Expectorated sputum assistance techniques				
Provider training and observed sputum collection	Malawian SN-TB suspects [41]: 39 out of 46 (85%) definite TB cases detected	Minimal staff training requirements Inexpensive Widely applicable in all settings	Time-consuming Infection control risk Not applicable for children	Programmatic research on implementation, uptake and efficacy
Sputum submission instructions/training	Pakistani females [42]: ↑ smear-positive case detection; ↓ spot-sputum saliva submission; ↑ females returning with sputum Indonesian males/females [43]: 15% higher case detection	Minimal staff training requirements Inexpensive Widely applicable in all settings No infection control risk	Time-consuming Not applicable for children	Programmatic research on implementation, uptake and efficacy
Sputum induction techniques				
Physical manoeuvres (*e.g.* chest physiotherapy)	Diagnostic yield (TB culture): adults, 5–26% [41, 44]; smear sensitivity (TB culture reference standard): adults, 50–53% [41, 44]	Safe procedure Minimal training/no equipment requirements	Low diagnostic yield High infection risk for health worker Currently restricted to hospitals with trained physiotherapists	No studies in primary care settings and children Few comparison studies with other methods of sputum induction
Ultrasonic nebulisation[¶]	Diagnostic yield (TB culture): adults, 8–34% [41, 45]; children, 10–30% [46, 47] Smear sensitivity (TB culture reference standard): adults, 37–78% [45, 48]; children[+], 20–57% [49, 50]	Safe procedure Noninvasive Feasible in resource-poor settings Good yield in adults and children Simpler performance in HIV-infected and -uninfected patients	Equipment and consumable costs High infection risk for health worker Currently restricted to district hospitals with infection control facilities	Few studies in primary care settings No studies of impact on patient-important outcomes, positioning in diagnostic algorithms and use of novel diagnostic tools on induced sputum samples
Other devices (*e.g.* vibration tool such as lung flute)	43% smear microscopy sensitivity [51] (small study of 15 patients)	Safe procedure Noninvasive Disposable/self-explanatory equipment decreases infection risk	High costs and waste of device Feasibility in different settings Infection risk Tools still in development	Prospective studies required in clinically appropriate settings
Alternative respiratory sample acquisition techniques				
Gastric washings[¶]	Diagnostic yield (TB culture): adults, 11–30% [52, 53]; children, 5–17% [46, 54] Smear sensitivity (TB culture reference standard): adults, 30–37% [41, 53]; children, 18–53% [46, 54]	Safe and effective procedure especially for children Minimal infection risk	Invasive procedure Requires fasting Sample collection advised on 3 consecutive days Not feasible in many public health facilities Currently restricted to district-level hospital settings	Programmatic studies of yield from resource-poor settings

Table 2. Continued.

Technique	Diagnostic performance ranges[#]	Advantages	Disadvantages	Knowledge gaps
Nasopharyngeal aspirate[¶]	Diagnostic yield (TB culture): children, 7–9% [54, 55] Smear sensitivity (TB culture reference standard): children, 58–71% [54, 56]	Safe Feasible across settings Useful for diagnosis of other respiratory pathogens (*e.g.* viruses), especially in children	Low diagnostic yield for TB Infection risk Currently restricted to district-level hospital	Programmatic studies of diagnostic utility in routine clinic settings
Bronchoscopy[¶]	Diagnostic yield (TB culture): adults, 9–46% [41, 57] Smear sensitivity (TB culture reference standard): adults, 27–63% [58, 59]	Equivalent diagnostic yield to other sample acquisition methods but allows direct visualisation of respiratory tract ± biopsy Allows diagnosis of other respiratory pathogens (*e.g. Pneumocystis*)	Invasive Requires specialised equipment and staff Restricted to tertiary and district hospitals Expensive Infection risk for health worker	Studies on the performance of novel diagnostics using bronchoalveolar lavage fluid to diagnosis or exclude active TB

SN: smear-negative. [#]: diagnostic performance characteristics are from prospective studies predominantly in high-burden countries. Wide variation and heterogeneity predominantly accounted for by differences in included study populations (*e.g.* SN-TB suspects *versus* SN-TB suspects with chest radiography suggestive of TB) and background TB prevalence. In induced sputum studies using ultrasonic nebulisation, adult studies include only SN/sputum-scarce TB suspects, while child studies are of TB suspects without prior diagnostic testing. Performance outcomes for novel diagnostics applied to acquired pulmonary samples are not included. [¶]: comparative studies of some/all of these sample acquisition techniques have been performed for adults [41, 44, 53, 58] and children [46, 54, 60]. For both adults and children, sputum induction using ultrasonic nebulisation is equivalent or superior to the more invasive techniques of gastric washing and bronchoscopy. [+]: a recent study of 452 children with suspected TB found diagnostic yield of induced sputum to be 15% and the sensitivity of the novel diagnostic, Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA), to be 83% (58 out of 70 patients) [61].

only 1–3%, decreasing to <1% after repeat testing, have been found across settings [14, 15]. Importantly, the use of MTB/RIF decreased the mean time to treatment initiation amongst smear-negative, culture-positive TB patients from 56 to 5 days, similar to that of smear-positive patients [14]. For the detection of rifampicin resistance, the meta-analysis data show a sensitivity and specificity of 94.1% and 97.0%, respectively [12]. On this evidence base, WHO has made a strong recommendation for the use of frontline Xpert® MTB/RIF in all patients with suspected drug-resistant (DR)-TB and/or co-infected with HIV, and a conditional recommendation, in acknowledgment of resource implications, for the use of Xpert® MTB/RIF as a follow-on test to microscopy in settings where multidrug-resistant (MDR)-TB or HIV is of lesser concern, especially for smear-negative TB [70].

Undoubtedly, a number of unanswered questions and concerns surrounding the use of the Xpert® MTB/RIF assay remain. First, although specificity for detecting rifampicin resistance remains >98%, studies continue to find false-positive rifampicin resistance results [75]. In areas of low MDR-TB prevalence, given the significant decrease in positive predictive value associated with small reductions in specificity, a large number of false-positive rifampicin resistance results may occur with widespread routine use. Despite the development of updated versions of both the GeneXpert® cartridge and software to further improve assay specificity, this remains an important concern. Secondly, given the reduced ability of a single Xpert® MTB/RIF test to rule out TB in HIV-infected patients [13], the role of additional Xpert® MTB/RIF tests and alternative investigations in HIV-infected patients with ongoing symptoms needs to be better defined. Thirdly, given that Xpert® MTB/RIF detects both viable and non-viable *M. tuberculosis*, the interpretation of a positive Xpert® MTB/RIF in patients not responding to TB therapy and the use, if any, of Xpert® MTB/RIF for treatment monitoring requires urgent clarification. Finally, a number of operational challenges and

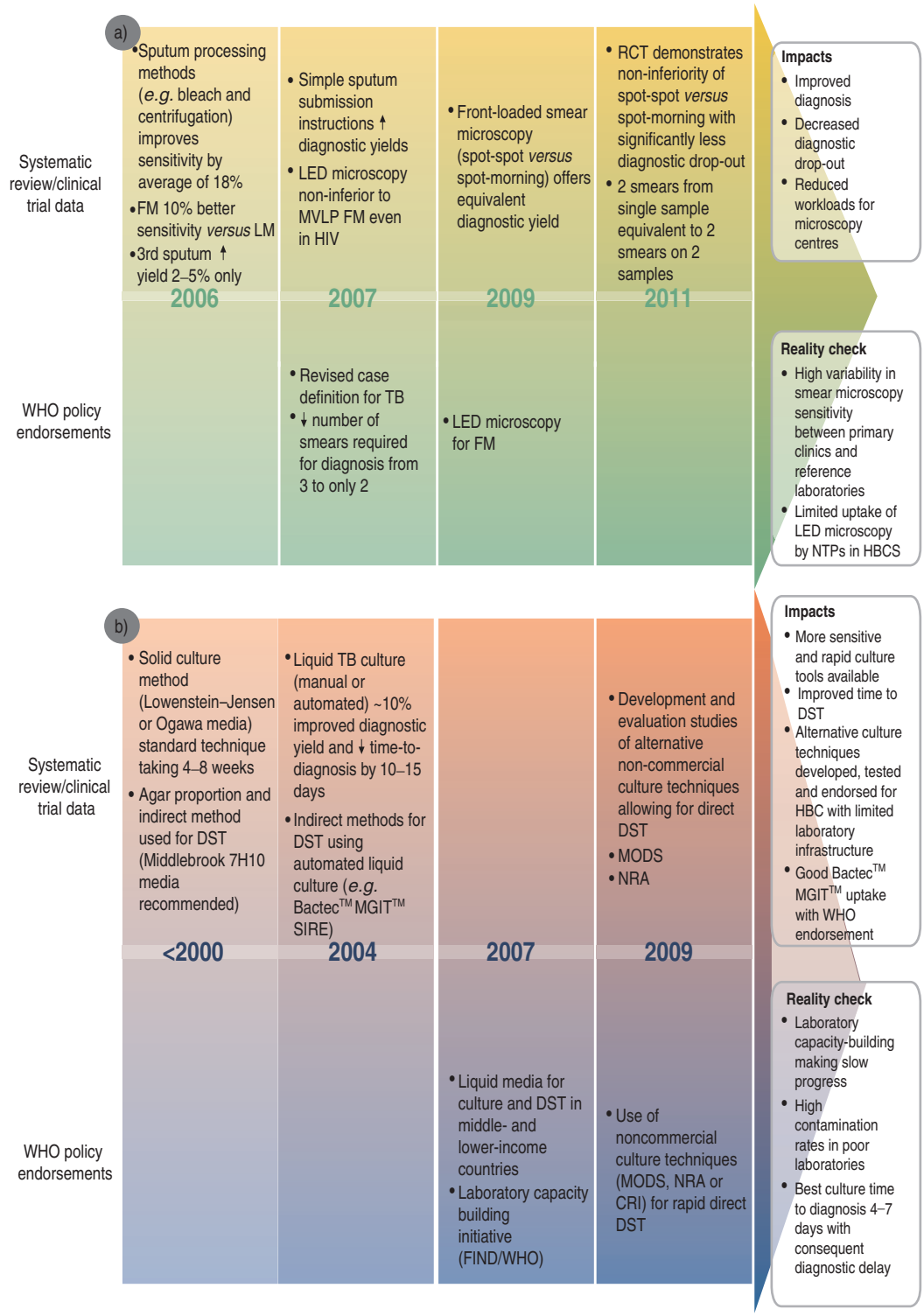
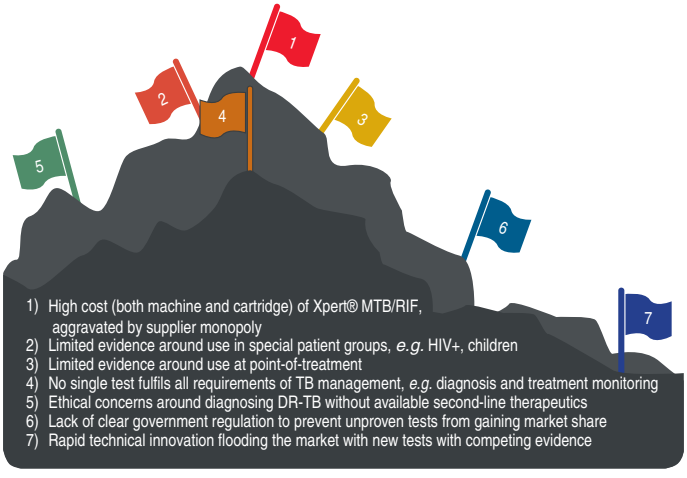

a)
Systematic review/clinical trial data
•Sputum processing methods (*e.g.* bleach and centrifugation) improves sensitivity by average of 18%
•FM 10% better sensitivity *versus* LM
•3rd sputum ↑ yield 2–5% only
2006
• Simple sputum submission instructions ↑ diagnostic yields
• LED microscopy non-inferior to MVLP FM even in HIV
2007
• Front-loaded smear microscopy (spot-spot *versus* spot-morning) offers equivalent diagnostic yield
2009
• RCT demonstrates non-inferiority of spot-spot *versus* spot-morning with significantly less diagnostic drop-out
• 2 smears from single sample equivalent to 2 smears on 2 samples
2011
WHO policy endorsements
• Revised case definition for TB
• ↓ number of smears required for diagnosis from 3 to only 2
• LED microscopy for FM
Impacts
• Improved diagnosis
• Decreased diagnostic drop-out
• Reduced workloads for microscopy centres
Reality check
• High variability in smear microscopy sensitivity between primary clinics and reference laboratories
• Limited uptake of LED microscopy by NTPs in HBCS
b)
Systematic review/clinical trial data
• Solid culture method (Lowenstein–Jensen or Ogawa media) standard technique taking 4–8 weeks
• Agar proportion and indirect method used for DST (Middlebrook 7H10 media recommended)
<2000
• Liquid TB culture (manual or automated) ~10% improved diagnostic yield and ↓ time-to-diagnosis by 10–15 days
• Indirect methods for DST using automated liquid culture (*e.g.* Bactec™ MGIT™ SIRE)
2004
2007
• Development and evaluation studies of alternative non-commercial culture techniques allowing for direct DST
• MODS
• NRA
2009
WHO policy endorsements
• Liquid media for culture and DST in middle- and lower-income countries
• Laboratory capacity building initiative (FIND/WHO)
• Use of noncommercial culture techniques (MODS, NRA or CRI) for rapid direct DST
Impacts
• More sensitive and rapid culture tools available
• Improved time to DST
• Alternative culture techniques developed, tested and endorsed for HBC with limited laboratory infrastructure
• Good Bactec™ MGIT™ uptake with WHO endorsement
Reality check
• Laboratory capacity-building making slow progress
• High contamination rates in poor laboratories
• Best culture time to diagnosis 4–7 days with consequent diagnostic delay

Figure 3. Progress in optimising and streamlining a) sputum smear microscopy and b) tuberculosis (TB) culture. Bactec™ MGIT™ and SIRE are manufactured by BD Diagnostics (Sparks, MD, USA). FM: fluorescence microscopy; LM: light microscopy; LED: light-emitting diode; MVLP: mercury vapour lamp; RCT: randomised controlled trial; WHO: World Health Organization; NTP: national TB programme; HBC: high-burden country; DST: drug-susceptibility testing; MODS: microscopy observed drug susceptibility; NRA: nitrate reductase assay; CRI: colorimetric redox indicator; FIND: Foundation for Innovative New Diagnostics.

research questions associated with the national and international scale-up of Xpert® MTB/RIF and other new TB diagnostic technologies remain and are outlined in figure 4.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1) High cost (both machine and cartridge) of Xpert® MTB/RIF, aggravated by supplier monopoly
2) Limited evidence around use in special patient groups, *e.g.* HIV+, children
3) Limited evidence around use at point-of-treatment
4) No single test fulfils all requirements of TB management, *e.g.* diagnosis and treatment monitoring
5) Ethical concerns around diagnosing DR-TB without available second-line therapeutics
6) Lack of clear government regulation to prevent unproven tests from gaining market share
7) Rapid technical innovation flooding the market with new tests with competing evidence

Figure 4. The challenges of scale-up and implementation of Xpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) and other novel tuberculosis (TB) diagnostic technologies. DR-TB: drug-resistant TB.

Line probe assays

As a rapid alternative to phenotypic DST, specialised NAATs using manual amplification and hybridisation techniques, known as line probe assays (LPAs) (table 1), offer *M. tuberculosis* speciation and genotypic DST with results in 1–2 days. LPAs received WHO endorsement in 2008 as the test of choice for rapid genotypic rifampicin and isoniazid DST [76]. LPAs offer sensitivities >98% for rifampicin resistance, but only ~85% for isoniazid resistance due to the presence of resistance coding mutations outside the regions of the *inhA* and *katG* genes detected by the assays [19, 77]. LPAs are now routinely available and are being scaled-up in certain national TB programmes, *e.g.* South Africa and India by the Expanding Access to New Diagnostics for TB (EXPAND-TB) project for MDR-TB suspects with smear- or culture-positive samples. Recently, an assay has been developed and is now available for rapid genotypic second-line DST and extensively drug-resistant (XDR)-TB diagnosis (GenoType® MTBDR*sl*; Hain Lifescience GmbH, Nehren, Germany). Sensitivities vary across initial studies depending on the specific drug tested [78–80] and clinical utility is restricted to rapidly ruling in XDR-TB at this stage.

Antigen detection

The detection of circulating TB antigens for diagnosis using different biological samples has been extensively studied [11]. A recent meta-analysis evaluated 47 studies using 12 different single or combinations of TB antigen [11]. Lipoarabinomannan (LAM), a 17.3-kDa immunogenic glycolipid component of the mycobacterial cell wall, is most extensively evaluated and, using the urine TB LAM ELISA (Alere, Waltham, MA, USA) (table 1), has shown promising utility for HIV-infected patients with advanced immunosuppression [81–84]. In HIV-infected patients, urine TB LAM ELISA has an overall sensitivity of ~50%, increasing to 67% and 85% in HIV-infected patients with CD4 counts <50 cells·mL^{-1} from out- and in-patient settings, respectively [82, 85], and an overall specificity of 83–100% [83, 86, 87]. Cross-reactivity with nontuberculous mycobacteria or other commensal organisms (*e.g. Candida* [83]) remains a concern. Given these performance characteristics, urine TB LAM ELISA, despite commercial availability, is not yet widely used or approved by WHO. However, urine LAM positivity has been correlated with bacterial burden [88] and may identify TB HIV co-infected patients with the highest mortality [89]. These factors, together with recent progression of the TB LAM ELISA into a POC lateral flow strip test, means that urine LAM, if used for rapid diagnosis to guide the early initiation of TB treatment in high-risk HIV/TB co-infected patients, may offer important clinical utility, and impact patient mortality and morbidity. Further research is necessary to confirm these hypothesised clinical benefits of POC LAM testing.

Immunodiagnosis of active TB

Numerous serological tests to detect TB-specific antibodies are available in many developing countries [90]. Updated meta-analyses show current serological assays are of no clinical value, with high variability in both sensitivity and specificity [10], and poor cost-effectiveness [91].

Despite the demonstrated lack of either accuracy or cost-efficacy, these tests continue to be sold in 17 out of 22 high-burden settings [90]. In India alone, an estimated US$15 million *per annum* is spent on performing serological tests for TB in the private sector [90]. In 2011, in response to this, WHO issued a negative policy advising against the use of any of the numerous available blood serological assays for the diagnosis of TB [92], and countries such as India have banned the clinical use of these tests. However, this policy does not discourage ongoing research into serological tests for TB diagnosis.

Blood-based interferon-γ release assays (IGRAs) in high-burden settings have also been extensively evaluated and found to offer little, if any, clinical utility as a frontline diagnostic tool for active TB in either HIV-infected or -uninfected patients [36, 93–95]. A recent WHO policy (2011) discourages use of IGRAs for active TB diagnosis in low- and middle-income countries [96]. The tuberculin skin test (TST) remains a useful tool for the diagnosis of active TB in young children and IGRAs offer equivalent, but not superior, performance [97, 98]. It is clear that immunodiagnosis is not a substitute for molecular or microbiological site-of-disease diagnosis, although its use for investigating LTBI remains important and is discussed later.

Diagnosis of TB infection

There is a growing recognition that LTBI is a spectrum that is poorly understood [99]. Both the TST and IGRAs are widely used as surrogate markers for TB infection and, consequently, for the diagnosis of LTBI. These assays have been extensively studied and systematically reviewed in a number of settings [94, 100–102].

IGRAs were developed to improve the specificity of TST, as they are not affected by bacille Calmette–Guérin (BCG) vaccination status. Hence, they are useful in the evaluation of LTBI

Table 3. Diagnostic accuracy of old and novel tests for common forms of extrapulmonary tuberculosis (EPTB)

Type of EPTB	Traditional diagnostic test performance	Commercial novel diagnostic test performance	Additional comments and/or research reference standards
TB meningitis	Smear: <5%; culture: 33–83%	Commercial NAAT: sensitivity, 56% (95% CI 46–66%); specificity, 98% (95% CI 97–99%) [110] Xpert® MTB/RIF: sensitivity, 29% (95% CI 4–71%) [111]; specificity, 100% (95% CI 82–100%) [111, 112]	Consensus guidelines of clinical case definitions and reference standards have been developed [25] Adequate sample collection and concentration may improve diagnosis (both for culture and Xpert® MTB/RIF) Use of combinations of diagnostic tests is an important area of ongoing research [117, 118]
TB lymphadenitis	Smear: up to 70% (HIV-infected); culture: 70–80%	Commercial NAAT [113]: sensitivity, 2–100%; specificity, 28–100% Xpert® MTB/RIF [111, 114]: sensitivity, 50–97%; specificity, 89–100%	Inoculation of aspirate or biopsy sample directly into culture bottles can improve yield In HIV-infected patients with disseminated disease, FNA lymph node is an important adjunct diagnostic tool and Xpert® MTB/RIF may offer rapid diagnosis
Pleural TB	Smear: <10%; culture: 12–70%	Commercial NAAT [115]: sensitivity, 62% (95% CI 43–77%); specificity, 98% (95% CI 96–98%) Xpert® MTB/RIF [111, 116]: sensitivity, 63% (95% CI 42–81%); specificity: 100% (95% CI 95–100%)	Pleural biopsy with histological/culture remains the reference standard Pleural fluid unstimulated IFN-γ has excellent preliminary diagnostic accuracy (lateral flow strip test in development for POC diagnosis)
Pericardial TB	Smear: <5%; culture: ~50%	Commercial NAAT: no data Xpert® MTB/RIF [111]: sensitivity, 68%; specificity, 89%	ADA biomarker, using a cut-point >30 IU·L^{-1}, shows sensitivity of 94% and specificity 89% Pericardial fluid unstimulated IFN-γ has excellent preliminary diagnostic accuracy (lateral flow strip test in development for POC diagnosis)
Abdominal TB	Ascitic fluid: smear, <5%; culture, 0–83%; bowel tissue: culture, 45–90%	Commercial NAAT: no data Xpert® MTB/RIF [112]: sensitivity, 29–100%	Novel diagnostics poorly studied in both ascitic fluid and histopathological samples

Xpert® MTB/RIF is manufactured by Cepheid (Sunnyvale, CA, USA). NAAT: nucleic acid amplification technique; FNA: fine-needle aspiration; IFN: interferon; POC: point-of-care; ADA: adenosine deaminase.

amongst BCG-vaccinated individuals, especially those who received BCG after infancy or multiple BCG vaccinations. Given the wide variations in BCG policies, online resources have been developed to guide practice by helping clinicians and public health practitioners review variation and their potential impacts on TST performance. These resources include a world atlas of BCG policy and practices [103], and a web-based algorithm for interpreting TST and IGRAs [104].

The use of IGRAs in clinical practice for diagnosing and managing LTBI shows considerable diversity. A recent survey of 33 IGRA guidelines and position papers from 25 countries and two supranational organisations has been published [101]. Four diagnostic approaches were commonly proposed: 1) a two-step approach of TST first, followed by IGRA either when the TST is negative (to increase sensitivity, mainly in immunocompromised individuals), or when the TST is positive (to increase specificity, mainly in BCG-vaccinated individuals); 2) either TST or IGRA, but not both; 3) IGRA and TST together (to increase sensitivity); and 4) IGRA only, replacing the TST. Overall, in low-burden settings, IGRAs are increasingly recommended to guide the use of preventative therapy. However, this survey suggests that most current guidelines do not use objective, transparent methods to grade evidence and recommendations, and do not disclose conflicts of interest [105].

Increasingly, it is becoming evident that neither IGRAs nor TST can adequately define or resolve the various stages of TB infection [99, 106]. A growing proportion of IGRA and TST studies show that both tests have limited prognostic value. For instance, a large proportion (>95%) of TST- or IGRA-positive individuals will not progress to active TB disease [107]. These findings indicate that the existing diagnosis of LTBI using IGRAs or TST may not be ideal to guide the use of preventive therapy to the subgroup of individuals who are most likely to benefit from it. Novel, highly predictive biomarkers, or combinations of biomarkers and risk factors (*i.e.* a composite risk prediction model), allowing for accurate prediction of patients with the highest risk of progression to active disease are urgently required [108]. For example, efforts are underway to develop a PCR-based test, as a follow-up test to IGRAs, for detecting transcriptional profiles of immune cells circulating in the blood, which might help predict risk of disease progression [109].

Diagnosis of EPTB

EPTB is diagnostically challenging and composite reference standards are the norm rather than the exception. Obtaining samples for diagnosis often requires specialised skills and equipment (*e.g.* biopsy and lumbar puncture), and the traditional diagnostics of smear microscopy and culture perform poorly on many of the paucibacillary, nonsputum biological samples.

Table 3 compares the performance of old and novel tools for the most common forms of EPTB. Overall, body cavity fluids (*e.g.* pleural, pericardial and cerebrospinal fluids) are paucibacillary, smear microscopy performance is dismal and liquid culture performs variably. Biomarkers, such as

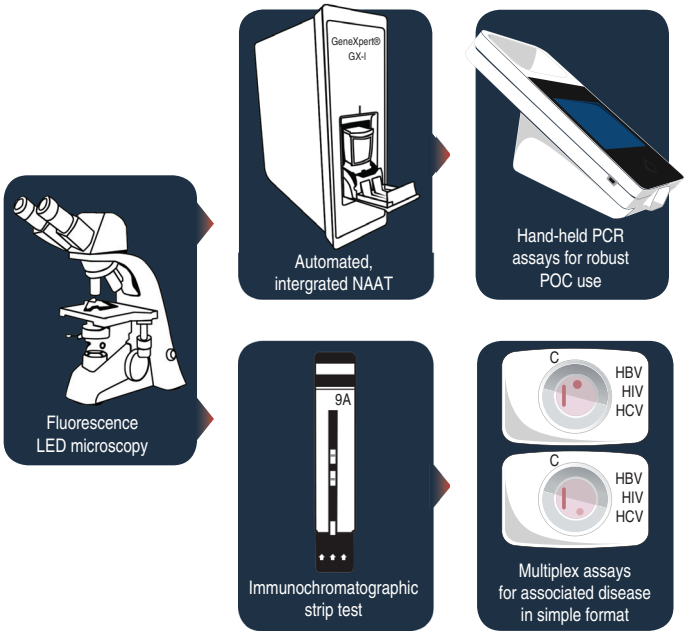

Figure 5. Current progress and future evolution of tuberculosis diagnosis from smear microscopy to molecular methods and onwards towards simple, affordable point-of-care (POC) test formats. GeneXpert® is manufactured by Cepheid (Sunnyvale, CA, USA). LED: light-emitting diode; NAAT: nucleic acid amplification technique; HBV: hepatitis B virus; HCV: hepatitis C virus.

unstimulated interferon (IFN)-γ and adenosine deaminase, appear to be useful but underused [119, 120]. Xpert® MTB/RIF looks promising for EPTB in initial studies with few patients and the benefit of concentrating larger samples volumes seem similar to culture, with minimal PCR inhibition ([111] and unpublished data).

Towards POC technology for active TB

Despite the advancement in the molecular diagnosis of TB and drug resistance, the need for a simple, instrument-free, laboratory-free POC test continues to be articulated by both research groups and civil societies [121–123]. Required minimum specifications for the ideal POC TB test have been defined by a number of groups and recently published [124]. Mathematical models suggest a huge potential impact of POC TB diagnosis on both case detection and overall TB incidence [125, 126].

Some commercially available novel diagnostic tests already come close to meeting these requirements and may offer important POC utility. The Xpert® MTB/RIF assay easily meets the specifications for diagnostic test accuracy (sensitivity >95% for smear-positive, culture-positive patients and 60–80% for smear-negative, culture-positive patients; specificity >95%) and time to result (<3 hours), but falls short as an ideal decentralised POC test because of its cost and the specialised equipment needed. Feasibility and impact studies of point-of-treatment, clinic-based Xpert® MTB/RIF are nearing completion and will provide insights on its POC utility.

For TB/HIV co-infected patients with advanced immunosuppression, the Determine® TB LAM Ag strip test (Alere) offers POC potential. It is an instrument-free, laboratory-free, affordable (<US$3.50) POC test producing results within 25 minutes and using an easily obtainable urine

Table 4. Important current unmet tuberculosis (TB) diagnostic needs and research gaps

Research gap and/or unmet diagnostic need	Rationale for need and/or research question(s)
1) Development of a simple, affordable, field-friendly POC for active TB using sputum samples	High-burden countries, severely limited resources Poor laboratory infrastructure and technical skills Patients have difficulty accessing health services and default prior to diagnosis
2) Impact evaluations of different simple and safe sample acquisition techniques *e.g.* sputum induction for sputum-scarce, smear-negative and childhood TB in primary care settings	Up to one-third of patients in high HIV and TB prevalence settings are unable to produce sputum All TB diagnosis relies on an adequate sample Sputum induction is simple and feasible yet carries high infection risk and moderate cost
3) Impact evaluations of Xpert® MTB/RIF at different healthcare levels, operational research and cost efficacy evaluations of Xpert® MTB/RIF, and optimal positioning of Xpert® MTB/RIF in diagnostic algorithms	Rapid WHO endorsement and plan for global implementation Benefits of a 2-hour test result may be lost if not used at point of treatment and rapidly available to patients Operational performance and actual cost efficacy unknown
4) Development of rapid, non-sputum based POC test for the diagnosis of EPTB and childhood TB	EPTB and children most often unable to produce sputum Biological samples (*e.g.* urine) readily available Certain forms of EPTB (*e.g.* TB meningitis) carry very high mortality and rapid diagnosis would save lives
5) Development of a rapid rule-out test for TB HIV co-infection for use in high-burden settings	TB can be clinically atypical in HIV co-infection but progresses rapidly with high mortality rate High TB drug-related morbidity in HIV-infected patients Other pathogens can mimic TB presentation and cause mortality if untreated
6) Further studies and impact evaluation of available POC urine LAM strip test for HIV-infected patients with advanced immunosuppression	First simple, affordable, rapid, non-sputum based TB diagnostic available Targets HIV co-infected patients with advanced immunosuppression and highest TB-related mortality Lack of clarity about test specificity, cut-point selection and test patient impact
6) Development of simple-to-perform, improved rapid molecular assays for first and second-line drug resistance	Growing epidemic of MDR- and XDR-TB All phenotypic DST methods require at least 10–14 days to provide results
7) Predictive biomarker(s) to identify latently infected people likely to progress to active TB and who will benefit most from preventive therapy	IGRA and TST predict progression to active TB suboptimally Isoniazid preventative therapy can cause significant individual morbidity and require large public health expenditure

Xpert® MTB/RIF is manufactured by Cepheid (Sunnyvale, CA, USA). POC: point-of-care; EPTB: extrapulmonary TB; LAM: lipoarabinomannan; WHO: World Health Organization; MDR: multidrug-resistant; XDR: extensively drug-resistant; DST: drug-susceptibility testing; IGRA: interferon-γ release assay; TST: tuberculin skin test.

sample with low infectious risk [82]. Unfortunately, diagnostic accuracy is dismal in unselected TB patients, and its use is restricted to HIV-infected patients with advanced immunosuppression [82]. Nevertheless, two initial evaluations in HIV-infected out- and in-patients showed similar diagnostic accuracy measures to the preceding TB LAM ELISA, and improved sensitivity when combined with sputum smear microscopy [127, 128]. Issues of test specificity and inter-reader agreement when interpreting the faintest bands still requires further study, while the measurable impact of a POC test with modest sensitivity on morbidity, mortality and hospital length of stay needs to be demonstrated prior to widespread uptake and WHO endorsement. Neither of these tests provide the ideal POC TB test, yet they indicate that the development of such a test may be within reach [121].

The ongoing progress towards POC TB diagnosis is outlined in figure 5. The diagnostic pipeline has many promising molecular POC tests and platforms under development. Hand-held or portable platforms using DNA chips and/or disposable cartridges are being evaluated for POC, simplified NAATs [122], while technologies to transition ELISA assays into simplified lateral flow POC test formats are well established and are being increasingly exploited. Although currently commercial serological tests are inaccurate for TB diagnosis, the detection of individual or combinations of TB-specific antibodies, antigens and or immune markers using lateral flow assays or microfluidic technologies still seems most likely to provide a field-friendly POC tool [122, 129]. In addition, both platforms seem to be evolving toward simultaneous detection and diagnosis of different infectious disease. Finally, electronic nose technology allowing analysis of breath condensates and the detection of distinct profiles of volatile organic compounds offers another possibility for POC TB diagnosis [130, 131].

Unmet needs and research priorities

Social, environmental, host and pathogen-specific factors continue to create distinct diagnostic challenges and settings (fig. 1), both at individual patient and public health levels. No single test has yet met, or perhaps will ever meet, all diagnostic requirements across resource, healthcare and clinical settings. Integration of old and novel technologies, and continued tailoring of technology to individual high- and low-burden, local and national settings is essential to optimise TB diagnosis. Table 4 highlights many of the current unmet diagnostic needs and research gaps. Ongoing basic and clinical research, as well as increased operational research, will be required to address these gaps. In particular, research moving beyond the simple assessment of diagnostic accuracy towards impact evaluations of novel tools and integrated algorithms for important patient and public health outcomes, such as morbidity, mortality, case detection

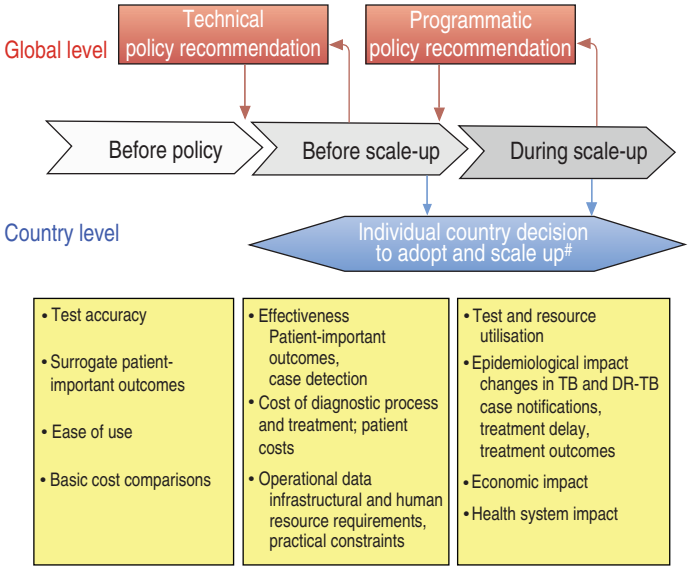
Global level
Technical policy recommendation
Programmatic policy recommendation
Before policy
Before scale-up
During scale-up
Country level
Individual country decision to adopt and scale up#
• Test accuracy
• Surrogate patient-important outcomes
• Ease of use
• Basic cost comparisons
• Effectiveness Patient-important outcomes, case detection
• Cost of diagnostic process and treatment; patient costs
• Operational data infrastructural and human resource requirements, practical constraints
• Test and resource utilisation
• Epidemiological impact changes in TB and DR-TB case notifications, treatment delay, treatment outcomes
• Economic impact
• Health system impact

Figure 6. Proposed new value chain for phased evaluation of tuberculosis (TB) diagnostics, from accuracy to impact assessment. Grey arrows: stages in the evaluation pathway; coloured boxes: policy decisions at the global level (red) and the country level (blue). In the stages before scale-up and during and after scale-up, evaluation data would be collected on diagnostic algorithms incorporating the new test. DR: drug-resistant. #: countries would adopt implementation at different points and should provide feedback about their experiences. Reproduced from [132] with permission from the publisher.

rates and/or default rates, and hospital length of stay [132], are required to best develop and guide policy. Recently, COBELENS *et al.* [132] proposed a new phased evaluation pathway for TB diagnostics (fig. 6).

Conclusion

The armamentarium of diagnostics tests for TB has never been greater. Nevertheless, many diagnostic challenges on both an individual patient level, such as for smear-negative or sputum-scarce TB, EPTB, TB/HIV co-infection and childhood TB, and on a larger public health level remain suboptimally addressed.

In addition, it is becoming increasingly evident that simply developing new tests will be insufficient to ensure successful scale-up and/or guarantee impact for either individual patients or the global TB epidemic [132]. Countries vary in their ability to embrace and in their interest in adopting new technologies, and it is clear that the willingness of national TB control programmes and private sector clinicians to use and invest in new TB diagnostics is fundamental to the successful widespread implementation of a novel technology. Even tools with excellent diagnostic accuracy, such as Xpert® MTB/RIF, may have little impact unless widely and appropriately used.

Thus, is it important that high TB-burden countries, especially the emerging economies (Brazil, Russia, India, China and South Africa), all with large TB and drug-resistance problems, drive the early adoption and scale-up of new technologies as well as lead the next wave of TB diagnostic innovation towards an affordable, simple POC test. In fact, only the combination of these efforts will allow advancements in TB diagnosis to significantly impact the global TB epidemic.

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Chapter 11

Omics and single molecule detection: the future of TB diagnostics

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SUMMARY: The diagnosis of tuberculosis (TB) depends on sputum-smear examination, mycobacterial culture and tuberculin skin testing (TST). Future diagnosis requires near-patient tests and early recognition of drug-resistant (DR) TB. Nucleic acid amplification techniques (NAAT) can detect approximately 100 bacilli·mL^{-1} and have moved from the laboratory into the field. Sensitivity can be improved by detecting proteins, using mass spectrometry, and their function, using reporter enzymes. Ultimately, microfluidics will permit reactions in restricted spaces with the potential to measure single molecules or sequence strains of *Mycobacterium tuberculosis* directly. Metabolic products released by TB and the human response to infection can be described by a characteristic metabolome and volatome of exhaled air. Interferon-γ release assays (IGRA) are blood tests that indicate the immune response to the few bacilli responsible for latent TB infection (LTBI). Their sensitivity may be improved by cytokines released by activated macrophages (inducible protein-10). The pattern of the immune response to many or all TB antigens, the immunome, may in future distinguish between active disease and latent infection.

KEYWORDS: Diagnosis, immunome, interferon-γ release assay, microfluidics, tuberculosis, volatome

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The Global Plan to Stop TB 2011–2015 [1] summarises the current state of play with respect to new diagnostic tests and processes involved in TB detection. Same-day sputum collection with immediate examination improves diagnosis and identifies those who need tuberculosis (TB) treatment [2]. Fluorescent microscopy using a light-emitting diode (LED) has a greater sensitivity for sputum-smear examination than Ziehl–Nielsen staining [3]. Liquid culture with rapid species identification is widespread. Microscopic observation of drug susceptibility (MODS) is a less expensive method of drug-susceptibility testing (DST) [4]. Line probe assays are being more commonly used to identify significant drug resistance at an early stage. The real-time nucleic acid amplification techniques (NAATs) to identify TB and suggest rifampicin resistance are being evaluated [5]. The aim is to identify those with active TB at the time and place that they present as TB suspects.

Our review will consider refinement and extension of current methods for testing, point-of-care testing, and new technologies. The benefits will vary according to cost, likelihood of reducing transmission or morbidity and whether the test is being considered as a screening tool or for the diagnosis of active disease.

Active TB

The key descriptor of new diagnostic tests is the limit of detection (fig. 1). Diagnostic tests in the past relied on culture to increase the number of bacilli. Current tests are based on NAATs or the detection of specific molecules (table 1).

DNA detection

DNA is most commonly detected by using polymerase chain reaction (PCR) technology. Loop-mediated isothermic amplification (LAMP) requires a single reaction temperature [12], and is less sensitive to inhibitors compared with standard PCR [13]. The detection of small quantities of DNA is already feasible. One technique is to attach a gold molecule to the sensing DNA strand, which appears red when on a single strand of DNA and blue-purple when complementary binding occurs [14]. A diagnostic DNA strand can also be attached to diamond nanowires, which will give a different electrical signal if the single stranded DNA detector binds its complementary strand [15]. These technologies have the promise of improving the limit of detection substantially.

Recombinase polymerase amplification (RPA) is a novel promising technology for NAATs testing. Recombinases are enzymes enabling primer binding for DNA amplification without thermal or chemical melting. The reaction can take place at temperatures from 22°C to 45°C and amplification from a few target copies to detectable levels occurs within 5–10 minutes [16, 17]. The RPA reaction system is stable as a dried formulation and reactions can be read using a portable real-time fluorometer (TwistDx Limited, Cambridge, UK). The enhanced precision of the primer pairing with the target DNA using a recombinase permits simultaneous assays with several primers (multiplex PCR) [18].

The use of NAATs to detect drug resistance implies a complete knowledge of all mutations that might be pertinent to therapeutic management. Recent interest in the problem of extensively drug-resistant

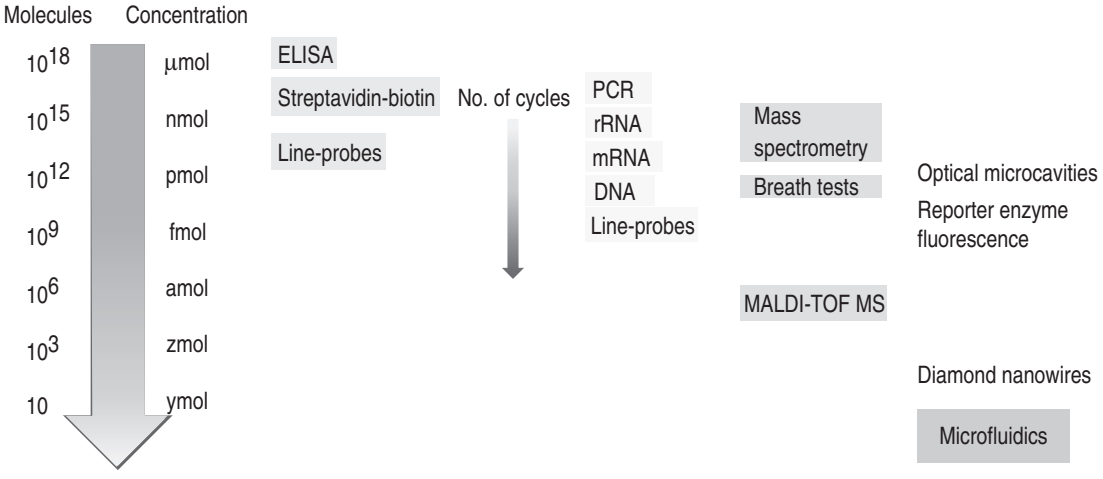

Figure 1. Sensitivity of diagnostic tests. MALDI-TOF MS: matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry.

Table 1. Current assays and limits of detection

Material	Method of amplification	Methods of detection	Time to result	Limits of sensitivity
TB bacilli	None	Ziehl–Nielsen stain	1–2 h	10^{3-4} bacilli·mL^{-1}
	Concentrated sputum	Fluorochrome	2 h	10^{3} bacilli·mL^{-1}
	Culture			10^{2} bacilli·mL^{-1}¶
	Lowenstein–Jensen	Colonies	6 weeks	
	MODS		2 weeks	
	Liquid culture	Turbidity/carbon dioxide	2 weeks	
rRNA 3/cell [6]	PCR	Real-time PCR probe hybridisation	2–48 h	10^{2} bacilli·mL^{-1}
mRNA	Reverse transcriptase PCR	Probe hybridisation	<48 h	Not used
DNA				
IS6110 0–30/cell	Needs culture+	Restriction enzymes	<48 h	10^{2} bacilli·mL^{-1}
MIRU-VNTR 41 [7]			<48 h	Not used for diagnosis
GeneXpert® MTB/RIF#		Probe hybridisation	4 h	10^{2-3} bacilli·mL^{-1}
Others		Line probes	2 h after culture	10^{2} bacilli·mL^{-1}
		Diamond nanowires		Not evaluated
Proteins	Culture	MS	<48 h	10^{2} bacilli·mL^{-1}
	None	MALDI-TOF MS	2 h	10 bacilli [8]
Metabolic products	Culture	MALDI-TOF MS	2 h	10^{2}·mL^{-1}
	None	Breath tests#	15 min	10 bacilli·nmole·L^{-1} [8]
Antigens				
PE/PPE 68 genes	Immune response	Antibody levels	4 h	Smear-positive disease 10^{3-4} bacilli·mL^{-1}
Species-restricted antigens		Antibody levels		Latent TB [9,10]
		Competition assays epitope-specific assays	4 h	Latent TB
ESAT-6/CFP10 DosR antigens		Interferon-γ		
		Supernatants	16 h	
		Immunospots	16 h	

TB: tuberculosis; IS: insertion sequence; MIRU-VNTR: mycobacterial interspersed repeating units-variable number of tandem repeats; GeneXpert® MTB/RIF: the GeneXpert® diagnostic kit for detecting *Mycobacterium tuberculosis* and resistance to rifampicin (manufactured by Cepheid, Sunnyvale, CA, USA). PE/PPE: Pro-Glu/Pro-Pro-Glu protein families of *M. tuberculosis*; ESAT-6: early secretory antigen target-6; CFP-10: culture-filtrate protein 10; MODS: microscopic observation of drug susceptibility; MS: mass spectrometry; MALDI-TOF; matrix-assisted laser desorption/ionisation time-of-flight. #: near-patient tests; ¶: liquid culture can detect as few as two bacilli, but not consistently [11]; +: the test cannot be performed directly on sputum but only on cultured organisms.

(XDR)-TB has highlighted the role of efflux activity [19, 20]. The future lies in the sequencing of respiratory pathogens, which has been accomplished using a microfluidic chamber [21].

Reporter enzymes

Reporter enzyme fluorescence (REF) technology detects bacterial enzyme products at 1,000-fold lower protein levels than detection of fluorescent proteins. *Mycobacterium tuberculosis* expresses a β-lactamase enzyme (BlaC), with a unique substrate binding site that allows for binding of specific substrates not catalysed by β-lactamases from other bacteria [22]. The key challenge is the development of specific and rapidly catabolised substrates; the current detection limit is 6×10^{2} colony forming units (cfu) with a 24-hour assay time. A low-cost point-of-care test called the μ-Lab™ TB POC-F assay is being co-developed by Global BioDiagnostics (Temple, TX, USA) and

the Foundation for Innovative New Diagnostics (FIND), the assay is expected to have diagnostic accuracy that is comparable with culture diagnostics.

Mass spectrometry

Matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry uses an aromatic matrix that absorbs the wavelength of the laser flux and can form microcrystals with the sample, which then sublimes at a low temperature enabling it to take part in a photochemical reaction that will ionise, but not decompose, peptides and proteins. The ionised peptides enter the gaseous phase directly and, because of their ionisation, take a measured amount of time relative to their mass to arrive at a detector. The matrix acts as a solvent for the sample and also absorbs the photon energy from the laser. An ion mirror can compensate for similarly charged ions having different energies and improve resolution and accuracy in the measurement of the relative molecular mass. Each species of bacteria and even different strains will give a different "fingerprint" [23], as shown with the detection of *M. tuberculosis* [8, 24]. MALDI-TOF mass spectrometry can distinguish smear-positive from smear-negative [25] and different forms of extrapulmonary (EP)TB [26] in a similar manner.

Microfluidics (lab on a chip)

Miniaturised nano/microfluidic platforms can manipulate small volumes of a fluid so that one or more chemical reactions can be undertaken with small quantities of biological material. These might include cell lysis, DNA extraction, PCR to amplify a sequence of interest followed by a signal to confirm that the sequence has been identified [27]. To date this technology has been used for diagnosis [28] and rapid strain typing of *M. tuberculosis* [29].The mass-production of such platforms using a desktop plotter, paper, poly(dimethylsiloxane) diluted in hexane [30] is an exciting advance.

Point of care

The incidence of TB could be reduced by 13–42% by 2050, using a point-of-care test [31]. Since 2004, the World Health Organization (WHO) has recommended that new diagnostic tests for TB should be affordable, sensitive, specific, user friendly, rapid and robust, equipment-free and deliverable (ASSURED) to users in the field [32].

Xpert® MTB/RIF

Several problems exist with transferring NAATs to a near-patient test: a specialised laboratory is often required; sample preparation can be difficult; the time taken for a test may be longer than the TB suspect is prepared to endure; and most tests need a supply of electricity. TB most often occurs in areas of the world with poor access to laboratories. Therefore, much excitement has been generated following the WHO endorsement of a near-patient diagnosis of TB and rifampicin resistance by NAAT directly on sputum in a contained environment. Initial validation of the Xpert® MTB/RIF (Cepheid, Inc., Sunnyvale, CA, USA), FIND and US National Institutes of Health (NIH)) system indicated that the level of detection was 131 cfu·mL^{-1} and that most of the common *rpoB* mutations that lead to rifampicin resistance could be detected [33]. In laboratory-based examination of sputum samples, the sensitivity of the test was 98.2% and 72.5% for smear-positive and smear-negative culture-positive pulmonary TB tests, respectively [34] and 69% for tissue samples [35]. The test was used successfully in routine laboratories in high-incidence countries with significant HIV co-infection [5]. However, the sensitivity was poorer in those without a positive smear and there were almost as many false-positive tests for rifampicin resistance as true cases when used in a clinic for those with a new diagnosis of HIV with a TB prevalence of 17.3% [36]. The positive predictive value (PPV) will inevitably fall as the incidence of TB in the population studied falls [37]. The near-patient use of this tool in the field requires

evaluation. Future developments include hand-held devices for NAATs that require very little power [38–40].

NAATs without electricity

PCR cycles involve DNA-strand separation, primer binding, primer extension and DNA synthesis. Heat can be generated by chemical reactions, such as the use of quick lime (CaO) and water. A prototype using LAMP and the same heat generation method has been developed for an isothermal reaction at 65°C to detect malaria [41] and a similar method could be used for TB [42].

Breath tests

In 1971, PAULING *et al.* [43] suggested that quantitative analysis of urine vapour and breath might have diagnostic potential. From an initial estimate of less than 300 different volatile organic chemicals (VOCs), more than 3,000 are now documented [44]. Lung biomarkers include nitric oxide (NO), inflammatory indicators related to oxidative stress, such as hydrogen peroxide (H_2O_2) and isoprostane, other nitrogen oxides, metabolites of arachidonic acid and cytokines. Bacterial products have been identified. The combination of host and pathogen VOCs should be of greater diagnostic merit than either host or pathogen VOCs alone. In TB, progress has been made towards identifying patterns that indicate active pulmonary disease, concentrating VOCs from large samples taken from end-alveolar air [45, 46]. The main problems remain the specificity of the VOCs and the level of detection, which should be of the order of one part in a trillion (picomolar).

Urine

A single study has suggested that patients with TB can be distinguished from healthy controls by a rise in *o*-xylene and isopropyl acetate with reduced 3-pentanol, dimethylstyrene and cymol in their urine [47]. Lipoarabinomannan (LAM) is one of the constituents of the cell well of *M. tuberculosis*. Serological responses to LAM have given poor specificity and sensitivity [48]. Detection of LAM antigen in urine also gave poor sensitivity and specificity [49], but may have some diagnostic merit in patients with HIV co-infection in those with low CD4 counts [50–52]. In active TB, small (180 base pair) fragments of bacterial DNA have been detected by PCR in the urine of patients with smear-positive pulmonary disease [53].

Pattern recognition and the immune response

Plasma has approximately 3,000 individual proteins, of which 20 represent more than 98% by mass [54]. Comparing material from TB patients with healthy controls, serum amyloid A, transthyretin, neopterin, and C-reactive protein gave a prospective diagnostic accuracy of 78% in patients with respiratory symptoms [55]. Physicians will immediately be sceptical that such nonspecific assays could contribute to the diagnosis of TB, but their availability and relative lack of cost mean that an evaluation as to whether a combination of nonspecific test can lead to a specific diagnosis should be investigated. Examples of pattern recognition and the immune response are given in table 2.

The pattern of antigens may be of more significance in identifying TB. As the microarray elements in microfluidic constructs become smaller, larger numbers of simultaneous tests can only be resolved with difficulty. Simultaneous detection of a sandwich immunoassay (capturing antibody stamped on a solid substrate, antigen and detector antibody) can be performed by attaching a gold nanoparticle to the detector antibody which catalyses the deposition of silver grains, whose reflections can be picked up by the pick-up head of a CD player [72]. Sandwich immunoassays using a magnet attached to avidin for a standard streptavidin-biotin amplification signal can be used to improve detection levels. Using such an approach, GASTER *et al.* [73] were able to perform as many as 64 assays on the same device. The analysis of B- [60] and T-cell [61, 62] epitopes may also be used as ''pattern recognition'' for the diagnosis of active and latent TB. For instance, the mass spectrometry of culture filtrate fractions with high interferon (IFN)-γ production suggests

Table 2. Examples of pattern recognition with potential utility in diagnostic tests

	Mycobacterium tuberculosis [ref.]	Human host [ref.]
Genome	Strain typing IS6110, MIRU-VNTR[#] Efflux genes [12, 13][¶]	Susceptibility[+] *e.g.* HLA-DR15 [56–58]
Transcriptome	Dormancy regulon [59] Genes expressed within the macrophage Persisters [60]	Innate immunity [61]
Proteome	MALDI-TOF MS species/strain identification [10, 11, 22, 50, 51] Smear-positive pulmonary TB [62] Phagosome protein expression [63]	CRP, serum amyloid protein, transthyretin, neopterin [53] Smear-positive pulmonary TB [56]
Immunome	Immunogenic secreted proteins [64] Potential cytotoxic T-cell epitopes [65]	Antibody levels to matrix-expressed TB proteins [66–68] Cytokine responses to expressed proteins [69, 70] Dendritic cell expression [71]
Metabolome	Reporter enzyme fluorescence, *e.g.* β-lactamase (*blaC*) [19]	
Volatome	Methylated n-alkanes, naphthalene and benzene [39–40]	Breakdown products of prostaglandins and other inflammatory markers [39, 40]

IS: insertion sequence; MIRU-VNTR: Mycobacterial interspersed repetitive units–variable number of tandem repeats; HLA–DR15: human leukocyte antigen-DR15; MALDI-TOF MS: matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. TB: tuberculosis; CRP: C-reactive protein. [#]: IS6110 is a piece of DNA that has the ability to replicate and insert itself at different points in the mycobacterial genome. MIRU-VNTR are silent genetic variations that can distinguish different strains of *Mycobacterium tuberculosis* and thereby might identify more transmissible strains. [¶]: limit development of drug resistance; [+]: limit use of preventive treatment to susceptible individuals.

that two new antigens (AcpM and PpiA), with greater recognition than known immunodominant antigens, such as early secretory antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10, can be identified [63]. Using a panel of 71 immunodominant antigens, differential expression of IFN-γ, interleukin (IL)-10 and tumour necrosis factor (TNF)-α were noted for a subset of six proteins during treatment, suggesting that these might be useful for monitoring anti-TB chemotherapy [64].

This approach has also been used to characterise the human innate immune response using transcriptome analysis [26]. A transcript signature of 393 mRNAs identified TB and subsets that could distinguish active from latent TB, as well as a group of latent TB with some transcripts noted especially in active TB.

Latent TB infection and screening tools

Cytokines

The immune response itself can be used to amplify the signal for detection in latent TB. IFN-γ release assays (IGRAs) have improved the diagnostic specificity in bacille Calmette–Guérin (BCG) vaccinated individuals compared with the tuberculin skin test (TST), but the tests cannot be used to differentiate between active and latent TB infection (LTBI), and the sensitivity is insufficient (~80%) to reliably rule out infection [74, 75].

The chemokine IFN-γ inducible protein (IP)-10 (CXCL10), and other effectors downstream from the IFN-γ signal can be used as alternative biomarkers to the classic T-cell cytokines [76–78]. IP-10 is expressed at very low levels in the basal state but at very high levels ($ng \cdot mL^{-1}$) upon macrophage activation. The diagnostic accuracy of IP-10 appears to be comparable with IGRAs and less influenced by low CD4 count or age [79]. Reverse transcription-quantitative nucleic acid amplification, which measures mRNA levels following antigen stimulation and can use just a

finger-prick volume of blood, is a promising tool for detecting IP-10 [80, 81]. IP-10 can also be detected by lateral-flow techniques in supernatants from QuantiFERON-IT (personal communication; B. Lange, D. Wagner, University of Freiburg, Department of Medicine, Division of Infectious Diseases, Freiburg, Germany) or in blood samples dried on filter paper [82]. IP-10 can be combined with IFN-γ for additional diagnostic sensitivity [63].

Immune responses to latency antigen Rv2628 of the *DosR* regulon have been associated with cured TB [55], although Rv2628-specific T-cell responses can be detected in the bronchoalveolar lavage (BAL) of patients with active TB presenting a peculiar phenotype characterised by effector memory cells [83]. Rv3407, a resuscitation protein involved in the return to active division after a period of dormancy, is one of 36 antigens, which distinguishes *M. tuberculosis* from *Mycobacterium bovis* BCG and was shown to boost BCG efficacy in a mouse model [84]. The same antigen was found to stimulate an IFN-γ response in those who had a positive TST and IFN-γ response to ESAT-6 and CFP-10 but did not have active disease [85].

Immunome analysis

Using transcriptome analysis, a subset of 86 mRNAs dominated by neutrophil-driven IFN-inducible genes and including both IFN-γ and type I IFN-α,β signals, could distinguish between active TB disease and LTBI [25]. Moreover, a subset of latent TB with features of some of the active TB transcripts was also identified. The creation of a diagnostic test from these data has yet to be realised.

Conclusions

Diagnostic tests and understanding of the pathogenesis of TB must go hand in hand. New technologies permit the detection of fewer and fewer molecules, but the definition of which molecules (antigens, peptides eliciting an immune response or the cytokines of the immune response itself) are of interest is the next most important step in future diagnostics for TB. Pattern recognition may become an important development in the diagnosis of TB.

Statement of Interest

M. Ruhwald is employed by Copenhagen University Hospital, Hvidovre and registered as inventor on issued and pending patents relating to the immunodiagnosis of infection with *Mycobacterium tuberculosis* using IP-10. D. Goletti has received a reimbursement from Cellestis to attend a scientific meeting in Croatia in 2009 and a partial travel reimbursement from Applicazioni diagnostiche avanzate to go to the American Thoracic Society (ATS) meeting in Canada in 2008. In addition the National Institute for Infectious Diseases L. Spallanzani (INMI) where D. Goletti works has received a grant from Oxford Immunotec as part of the salary for a laboratory technician in 2007.

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Chapter 12

Treatment of TB

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SUMMARY: The treatment of all forms of tuberculosis (TB) is based on two principles: 1) the combination of drugs (at least four) to avoid the selection of anti-TB drug resistances; and 2) the need for prolonged treatment in order to ensure that all bacteria in their different phases of metabolic growth are effectively killed. The selection of drugs to be included in the combination is based on their bactericidal and sterilising capacity, and their ability to prevent drug resistance. Following extensive research in the field in the second part of the last century, the optimal regimen of initial treatment of all forms of drug-sensitive TB, both pulmonary and extrapulmonary, is 2 months of isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E), followed by 4 months of treatment with H and R (2HRZE/4HR). The impossibility of using R, the most powerful of all anti-TB drugs, substantially complicates the treatment of TB.

In this chapter, we discuss the fundamental basis of anti-TB therapy, demonstrating that with proper clinical and operational management, patients with TB have a high probability of being cured, even though this probability is reduced with increasing levels of *Mycobacterium tuberculosis* drug resistance.

KEYWORDS: Anti-tuberculosis drugs, extensively drug-resistant tuberculosis, multidrug-resistant, multidrug-resistant tuberculosis, treatment, tuberculosis

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Until the advent of the antibiotic era, the prognosis of patients with tuberculosis (TB) was heart-breaking. In fact, the spontaneous cure rate was only 25–30% [1]. The prognosis began to change notably during 1950–1960, when well-designed clinical trials, using associations of the recently discovered antibiotics, began to show that TB could be cured in most cases [2]. These first clinical trials provided a fundamental understanding of the rationale of the treatment of all TB cases: either they carry strains of *Mycobacterium tuberculosis* susceptible to anti-TB drugs or they have some degree of antibiotic drug resistance.

The initial use of different anti-TB drugs as monotherapy resulted in a high sputum smear conversion rate and initial clinical improvement in the patients. However, the probability of relapse due to acquired anti-TB drug resistance on monotherapy was very high. In this way, the first and most important concept of TB treatment was identified: the need for a drug combination to prevent the selection of anti-TB drug resistance.

Those early years of experience also taught us that a significant percentage of relapses occurred if the treatment was discontinued at the time when clinical symptoms disappeared and sputum smears converted to negative. These observations led to the second major concept of TB treatment: prolonged treatment is necessary to eliminate the great majority of *M. tuberculosis* strains, some of them living in a microaerobic/anaerobic environment in an altered state of metabolism [2, 3].

At present, it is widely accepted that anti-TB chemotherapy should be based on these two important bacteriological considerations: the combination of drugs to avoid the development of resistance and the need for prolonged chemotherapy to prevent disease relapse. This applies to all forms of TB, regardless of the pattern of *M. tuberculosis* drug resistance. The number of drugs in a combination regimen and the total duration of treatment depend on the efficacy of the drugs in the regimen to cure the disease and to prevent relapses [3].

In this chapter, we review and discuss the bases that should guide the treatment of all forms of TB. If these rules are properly followed, it will be possible to design an effective treatment regimen in the great majority of cases, including patients presenting patterns of extensive *M. tuberculosis* drug resistance.

The need for drug combinations to prevent drug resistance selection: how many drugs are needed to treat TB?

Although all the bacilli present in a colony originate from a single cell, the bacteria do not show a homogeneous behaviour against the different anti-TB drugs. Spontaneous, natural mutants arise during successive bacillary divisions as a random event, including mutations in genes that carry antibiotic drug resistance. The probability of these mutations occurring is closely associated with the number of bacilli present. The selection of resistant mutants is due to drug selection pressure. On average, 10^5–10^6 bacilli are necessary for the appearance of a natural mutant resistant to each of the anti-TB drugs. These mutations are independent for each of the different drugs, as different genetic targets are involved. The probability that resistance to two drugs may develop simultaneously is equal to the product of their single respective mutation rates and practically impossible to observe in human TB. Cavitary and smear-positive TB present the highest bacterial load, which can reach up to10^9–10^{10} bacilli. This situation carries the highest risk for the selection of resistant mutants if monotherapy or inadequate combinations of drugs in polychemotherapy are given (*e.g.* when existing drug resistance of *M. tuberculosis* is not known or not considered). The risk is much lower in pulmonary TB with infiltrations but without cavitations (10^7–10^8 bacilli), and even lower in nodular pulmonary TB and extrapulmonary TB, where the bacillary load barely exceeds 10^5–10^6 organisms [3].

If monodrug treatment is started in a patient with cavitary pulmonary TB, initially, most of the bacilli will be eliminated by the bactericidal activity of the drug and the symptoms usually improve. However, in this initial phase, treatment selects bacteria that are resistant to the drug, which in a short time become the dominant microbial population. Signs and symptoms will then rise again (the so-called "fall and rise" phenomenon) [4]. In addition, the drug in question will have become useless for the treatment of the current episode of the disease.

Thus, all monotherapeutic regimens evaluated so far for the treatment of TB (real or masked by combination with drugs to which resistance has previously been established, or which prove ineffective) inevitably lead to treatment failure and to the development of drug resistance.

Therefore, if *M. tuberculosis* is fully susceptible to all of the anti-TB drugs, with no practical risk of the natural occurrence of resistance to two drugs (since the weight and volume to sustain this bacillary load would be too large for the human body: 10^{13} for isoniazid (H) plus rifampicin (R) and 10^{18} for H plus R plus ethambutol (E)) [3], the use of two very active drugs (H plus R) could cure practically all susceptible cases of TB.

Unfortunately, however, there are already a considerable number of *M. tuberculosis* strains being transmitted in the community with H or other drug resistance. This is why it is advised that E

should be systematically added to the the standard treatment regimen, a weak drug with little ability to kill, but with an extraordinary ability to protect R in case there is initial resistance to H. This leads to the need to use a minimum of three active drugs. Addition of pyrazinamide (Z) to the combination of HRE in the initial 2 months of treatment results in shortening the total duration of the treatment to 6 months. Treatment with Z is only beneficial in the first 2 months (intensive phase (IP)), while treatment with H and R should be continued for another 4 months (continuation phase (CP)). The ideal duration of E will be discussed later.

In summary, the first premise of any TB treatment is that of combining at least four drugs not used previously in a treatment regimen for this patient or those with a high likelihood of being effective. The matter of the correct drug combination in patients infected with drug-resistant (DR) strains of *M. tuberculosis* or in patients who experience adverse drug events will be reasoned out below.

The need for prolonged treatments: bactericidal *versus* sterilising activity of anti-TB drugs

The second premise for the TB treatment regimen is the need for an extended treatment duration in order to give the drugs the ability to kill *M. tuberculosis* in its different growth phases. The growth and metabolic activity of *M. tuberculosis*is are affected by the surrounding oxygen tension and pH. The ideal conditions for the bacillus comprise a pH of 7.40 and an oxygen tension of 110–140 mmHg. Based on the different characteristics of the environment in which *M. tuberculosis* is growing, three bacterial growth modalities have been established that condition the bases of the currently used drug combinations and the duration of treatment [3, 5]. These three bacterial populations are listed below.

Metabolically active mycobacteria under conditions of continuous growth

In patients with active TB, most of the bacilli (10^7–10^{10}) are metabolically active mycobacteria under conditions of continuous growth. They are located within the cavitary walls, where the conditions of oxygen pressure and pH are ideal for growth. The capacity of a treatment regimen to eliminate this particular bacillary population is referred to as bactericidal activity [3, 5]. A clinical surrogate marker for bactericidal activity is the percentage of patients with negative cultures at the end of the second month of treatment. Apart from the effectiveness of the therapy, the bactericidal activity of the drugs is fundamental to reduce the infectiousness of the patient. The bactericidal activity of all drugs with recognised activity against *M. tuberculosis* is listed in table 1. Drugs with higher bactericidal activity are preferred when designing the patient's treatment regimen. H and R have the best overall bactericidal activity. Among the second-line drugs (SLDs), only the fluoroquinolones (FQs) (especially new-generation) and the injectable drugs have good bactericidal activity. Thionamides have moderate bactericidal activity [3, 5].

Bacilli in the acid inhibition phase

Bacilli in the acid inhibition phase consistute a less numerous population (10^3–10^5 bacilli) because their growth is inhibited by a low pH medium. Bacteria in this group are either located intracellularly within phagolysosomes of macrophages or extracellularly in the inflammatory zones of cavitary walls. The limited oxygenation of the environment contributes to the inhibition of bacterial growth. Due to their altered metabolic activity, these bacteria are unlikely to be eliminated by the administered drugs. For this reason, this bacillary population, which is in a sporadic multiplication phase and generally referred to as the "persistent bacterial population", probably represents the main source of TB relapse [3, 5].

The capacity of drugs to eliminate this bacillary population (and the bacilli in sporadic multiplication phase; see later) is referred to as "sterilising activity". The sterilising activity can be quantified as the frequency of relapses that follow treatment completion. The sterilising capacity of

Table 1. Chemotherapy in tuberculosis

	Prevention of resistance	Bactericidal activity[#]	Sterilising activity[¶]	Toxicity
High	H E R Injectables[+] FQs[§] Cs/Trd PAS Pro/Eto	H E R Injectables[+] FQs[§]	Z R New FQs[§] Injectables[+] FQs[§]	Cs/Trd PAS Pro/Eto Injectables[+] Z
Moderate				
Low	Z	Pro/Eto Z	H	E H FQs[§] R

Activity of different World Health Organization (WHO) group I–IV drugs. Apart from a high toxicity profile of linezolid, reliable information for WHO group V drugs cannot be provided. H: isoniazid; E: ethambutol; R: rifampicin; FQ: fluoroquinolone; Cs: cycloserine; Trd: terizidone; PAS: para-aminosalicylic acid; Pro: prothionamide; Eto: ethionamide; Z: pyrazinamide. [#]: cycloserine/terizidone and PAS have practically no bactericidal activity; [¶]: ethambutol, cycloserine/terizidone, PAS and prothionamide/ethionamide have practically no sterilising activity; [+]: amikacin, capreomycin and kanamycin; [§]: ciprofloxacin and ofloxacin (old generation), and levofloxacin and moxifloxacin (new generation).

all the anti-TB drugs is also shown in table 1. The excellent sterilising capacity of Z has allowed reduction of the duration of treatment to 6 months. If Z is not included in the initial treatment phase, treatment must be extended to at least 9 months to give R the ability to kill slowly replicating mycobacteria [3, 5]. It is possible that the new FQs may also have a sterilising capacity on these bacilli.

Bacilli in a sporadic multiplication phase

Bacilli in a sporadic multiplication phase also constitute a limited population (10^3–10^5 bacilli), preferentially located in the solid caseum, where the pH is nearly neutral. These bacilli undergo long dormant periods with occasional and brief replication periods lasting hours. As a result, the administered medication is only able to destroy these bacteria during these periods of active metabolism, and such periods may not occur at any time in the course of therapy. However, the scant and occasional activity of these bacteria prevents them from developing resistance. The drug of choice for eliminating this population is R, fundamentally due to the rapid onset of its sterilising action (15–20 minutes *versus* 24 hours in the case of H) and the excellent tissue penetration [3, 5].

The drugs with the strongest sterilising action are R and Z (only capable of acting on the bacillary population described here), while H and E have a lower sterilising capacity. Among the SLDs, only new FQs seem to have a good sterilising action; this action is rather limited in streptomycin and other injectable SLDs, and may not exist in the rest of the SLDs. The greater the sterilising activity of drugs included in a treatment regimen, the more its duration can be shortened [3, 5].

For how long should TB be treated?

The total treatment duration depends on the presence of *M. tuberculosis* drug resistance, the combination of drugs, their tolerability and their mode of action (table 1). If R, the best drug currently available, can be included in the regimen, treatment duration may be reduced to 9 months, even to 6 months if Z can also be used in the initial phase of the treatment. However, whenever R cannot be used (due to adverse events or drug resistance) treatment regimens should not be less than 18 months, and could be even longer if H cannot be used either [3]. However, one recent study with multidrug-resistant (MDR)-TB patients has achieved excellent cure rates with a

treatment regimen of 9 months, very probably because of the high doses of a later-generation FQ used in the regimen [6]. FQs may end up playing a role similar to R in the future.

Core *versus* companion drugs: IP and CP

To our current knowledge and with currently available drugs, TB treatment should last 6 months or longer and should include a minimum of four drugs in the IP. Only in the case of known drug susceptibility to H plus R could three drugs (H plus R plus Z) be enough. Theoretically, only four drugs are needed in the IP when the bacillary load is very high. When bacillary load has been notably reduced, fewer drugs are needed. Two very active drugs that are responsible for killing and sterilising *M. tuberculosis* must be retained, if possible, throughout the entire treatment. The two other accompanying drugs that kill little, but protect the core drugs so that the bacillus does not develop resistance, can be stopped when shifting from the IP to the CP.

Following this reasoning, the ideal treatment regimen for TB should have two phases: 1) one IP with four new drugs (two core and two accompanying) until the bacillary load has been reduced to a minimum; and 2) the CP, where the accompanying drugs can be stopped. The best indicator that the bacillary load has been reduced to a minimum and that the IP can safely be replaced by the CP is the reversion of the sputum smear to negative. Although some prefer to use culture conversion as a proxy of bactericidal activity, most experts agree that sputum smear conversion is an appropriate proxy for bactericidal activity. Following sputum smear conversion, two potent drugs are likely to kill the remaining bacilli in the CP. In order to standardise the regimen, at least 2 months of IP is recommended for the initial TB cases without suspicion of DR-TB, in spite of an earlier smear negativisation. At least of 4 months of CP is also recommended, because the risk of relapse can increase if the combination therapy is provided for a shorter period of time.

Based on the mode of action of the drugs listed in table 1, the best existing core drugs for the CP are H and R. Other core drugs include later-generation FQs and injectables, which must be incorporated in the regimen when H or R cannot be used, until better drugs become available. Between the later core drugs, the FQs can also be given throughout treatment. However, injectables should be stopped at the end of the extended IP due to their very high cumulative toxicity. The remaining drugs, with the possible exception of thionamides, play practically no role as core drugs.

Ideal treatment regimen for new TB cases: daily *versus* intermittent therapy

The best treatment for TB caused by drug-susceptible strains of *M. tuberculosis* should include H, R and Z in the first 2 months, followed by H and R for another 4 months (2HREZ/4HR). E is also added to cover the possibility that a patient is infected by H-resistant TB bacilli (more than 10% of new TB patients worldwide). This treatment regimen offers potent bactericidal and sterilising action, curing more than 95–98% of the patients, with few relapses (less than 1–2%) and few adverse events (less than 5%). Z should only be administered for 2 months, since after this period, the great majority of infected phagolysosomes and extracellular compartments with acid pH conditions have disappeared (*i.e.* the conditions of preferential action for this drug). The sterilising action of Z may already be very scarce or nil after the second month of treatment if R is included in the regimen. In addition, as E is given to protect against the possibility that the patient has been infected by bacilli resistant to H, E should not be stopped until the *M. tuberculosis*-drug susceptibility pattern to all first-line drugs (FLDs) is known and susceptibility to H plus R is probed, or at least until the sputum smears have converted to negative. In fact, this would probably relate to a bacillary load that is so low that R alone would be able cure the patient, even in the event of initial resistance to H [7]. In this way, R will be always protected, and this should be the primary goal of all initial treatments advocated in TB programmes.

Although the CP with 4HR or until completing 6 months of treatment with HR is usually enough to cure most patients, a series of conditions have been identified in recent years that may facilitate relapses. In HIV infection, it is now widely accepted that the CP should be prolonged until completing 8–9 months of treatment with HR [8]. Other conditions, such as extensively advanced or cavitary TB, or those with a delayed smear and/or culture conversion, seem to benefit from prolongation of the CP [9]. Although standardised regimens should always be applied under control programme conditions, in patients with delayed bacteriological conversion, it may be advocated to maintain the CP for at least 4 months following sputum smear conversion.

Ideally, the entire treatment should be administered daily. There is evidence that the initial treatment may be equally effective if the drugs are administered two or three times a week, based on the good post-antibiotic effect (PAE) of H and R [10]. However, missing drug doses are important risk factors for treatment failure in intermittent therapy. In addition, the PAE of H is different from that of R. Therefore, the possible growth of the bacilli that are naturally resistant to each of these drugs is different [11]. While intermittent therapy cannot be advocated in the IP, treatment schemes that use daily treatment during the IP and treatment three times a week in the CP, may be advocated under special circumstances, when maximum patient adherence to treatment cannot be ensured [12].

In conclusion, the ideal initial treatment for all TB cases due to *M. tuberculosis* that is pan-drug sensitive is 2HRZE/4HR, with possible variations in the IP and CP, as discussed earlier.

Treatment of TB in special situations: extrapulmonary TB

The previously mentioned basic principles for the treatment of pulmonary TB also apply to all forms of extrapulmonary TB (EPTB), including some serious forms such as meningitis or miliary tuberculosis. Thus, the treatment should be identical, since none of the possible presentations of EPTB is associated with a greater bacterial load or a greater number of latent bacilli to justify modifications of the treatment [13].

However, this is a matter of controversy. While some international organisations defend the idea that treatment of EPTB should be the same as for pulmonary TB [14], other prestigious medical societies recommend longer treatment durations for some EPTB presentations, such as meningeal, miliary or osteoarticular TB [15]. However, randomised controlled trials have shown excellent outcomes with 2HREZ/4HR regimens in the treatment of lymph node and spinal TB, and other nonrandomised prospective and retrospective studies have also shown excellent outcomes with 2HREZ/4HR regimens for other forms of EPTB, including meningeal TB [13, 16–18]. Therefore, we recommend that the treatment of all forms of EPTB should be the same as that of pulmonary TB, with the possibility of extending its length in some severe forms of tuberculous meningitis, cerebral TB and osteoarticular TB.

Steroids are only indicated in the treatment of severe forms of TB, such as meningeal, miliary or pericardial TB, where their anti-inflammatory properties can be effective in the acute phase to reduce the sequelae [3, 14].

In HIV infection, it is now widely accepted that the continuation phase should be prolonged until completing 8–9 months of treatment with HR due the higher probability of relapse after treatment with a standard regimen [8]. However, international guidelines still state that the duration of treatment should not be extended in TB/HIV co-infected patients, as the benefit is unclear compared to the problems of creating a different standard of treatment for HIV-infected and -uninfected persons [14, 19]. These recommendations may change in the near future. Intermittent regimens should be avoided, at least in the IP [14, 19].

As for HIV-infected patients with TB, it is not necessary to modify the initial treatment regimens in individuals with other form of immune deficiencies, pregnant or nursing females, children or infants [14]. The only requirement is to adjust the corresponding dose and to ensure close follow-up. In the

case of malabsorption or if the patient is unable to take medication orally, the same regimens are provided, but *via* enteric feeding or the parenteral route [3].

In patients with hepatic impairment, a standard regimen without Z (the most hepatotoxic drug and the least relevant in the regimen) and a CP of up to 9 months may be advocated. In advanced liver disease, the possibility of using R in the regimen as the only hepatotoxic drug should be considered. If R cannot be used, a regimen with a later generation FQ, E and streptomycin (Sm) should form the pillar of treatment [3].

In patients with advanced kidney disease (*i.e.* with creatinine clearance below 30 $mL \cdot min^{-1}$), E, Z, ofloxacin, levofloxacin (Lfx), the injectable agents (amikacin (Am), capreomycin (Cm), kanamycin (Km) and Sm) and cycloserine (Cs) should only be given three times per week. Drugs with potential nephrotoxic effects, such as E and the injectable agents, should be avoided. If these drugs are used, blood drug levels should be monitored to ensure dose adjustment [3, 14].

Currently there is no clinical evidence available for the optimal monitoring intervals in patients with hepatic and/or renal impairment on TB treatment.

Ideal treatment regimen when R or H cannot be used: treatment of mono- or polydrug-resistant non-MDR-TB

DR-TB, especially when there is resistance to R, has become the main challenge to overcome in TB worldwide. In fact, R is by far the most active drug in the treatment of TB. R is the only anti-TB drug that meets the four major properties that should be expected from a drug with activity against this microorganism: bactericidal and sterilising capacity, ability to prevent resistance, and low toxicity (fig. 1). When it is not possible to use R for the treatment of patients with TB, either for resistance or intolerance, the treatment duration is prolonged to at least 18 months and the prognosis worsens, compared with patients who can be treated with R. Furthermore, resistance to this drug is relatively simple to detect using rapid molecular methods.

Patients with H mono- or polyresistance but who retain susceptibility to R are relatively common in all National TB Control Programmes (NTP), and it is an acceptable and credible situation in the field. They are relatively easy to treat patients. The combination therapy for H monodrug resistance advocated by the World Health Organization (WHO) currently includes R, E and Z over 9 months. In advanced cases, a later-generation FQ should be added [20]. In contrast to the WHO recommendations, the ideal combination therapy for a patient with H monoresistance could also include R, an FQ and E, with the initial support of Z during the first 2 months [3].

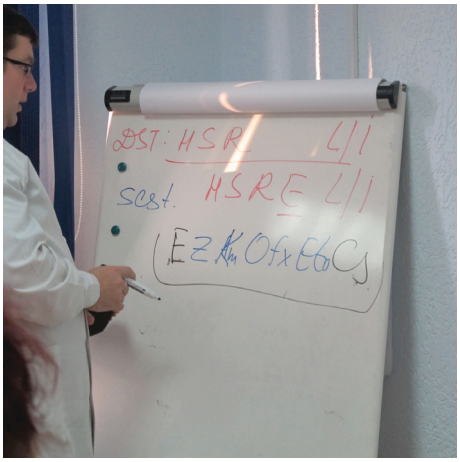

Figure 1. Designing a multidrug-resistant tuberculosis regimen during a consilium in Minsk, Belarus.

A completely different situation is that of *M. tuberculosis* with R mono- and polyresistance that retains susceptibility to H. This situation is very rare, as more than 90% of cases of R resistance are actually MDR. Moreover, it must be remembered that while the reliability of drug-susceptibility testing (DST) for H is high, it is not perfect (100%). Consequently, all R mono- or polyresistant TB cases must be managed like MDR-TB patients. Accordingly, an MDR-TB regimen would be designed following all the premises discussed in the next section. H should be included in the combination therapy but should preferably not be counted among the four drugs that must form the core of the treatment. In this way, the patient will have a high likelihood of being cured when the combination

therapy is administered over at least 18 months, whether *M. tuberculosis* is MDR, or is really R mono- or polyresistant but retains susceptibility to H.

Ideal treatment regimen when R and H cannot be used: treatment of MDR-TB

Of the remaining drugs active against *M. tuberculosis*, only the new FQs have a bactericidal and sterilising activity similar to R (although to a lesser extent than R), and similar low toxicity, but much less ability to prevent resistance than R. Therefore, drug resistance to FQs clearly influences the prognosis in extensively drug-resistant (XDR)-TB, defined as MDR-TB with additional resistance to any FQ and at least to one of the three injectable SLDs (Am, Cm and/or Km) [21, 22].

The treatment of MDR-TB or patients that cannot be treated with H and R due to adverse events follows the same recommendations as mentioned previously: at least four active drugs in the IP followed by an extended CP to guarantee a minimum rate of relapse [20, 23].

Theoretically, only three fully susceptible drugs could be sufficient to cure the patient and to prevent the development of anti-TB drug resistance [23]. However, it is recommended that at least four drugs are used in the combination because there is a risk that some of the strains transmitted in the community already carry some drug resistance. Of the four drugs, two should fulfil a "core" role.

In the patients with MDR-TB, the possibility that *M. tuberculosis* can be susceptible or resistant to a drug must be evaluated by considering the drugs the patient has taken in the past for the treatment of previous episodes of TB and the results of DST. The clinical reliability of DST is very good for H and R, and for FQ and SLD injectables, but is low for the remaining FLDs and SLDs [24–26].

The ideal progressive sequence for the introduction of anti-TB drugs and the recommended dosages are described in table 2. From each of group 2 (later-generation fluoroquinolones) and 3 (second-line injectable drugs), only one drug can be selected [20, 27].

In the treatment of patients with MDR-TB, the IP is defined by the duration of treatment with an injectable SLD (group 2 in table 2), which is generally recommended for a duration of 8 months [28, 29]. However, the level of evidence for this conditional recommendation is very low [28, 29]. For this reason, the length of administration of the injectable drug should be decided in the context of the potency of other drugs in the regimen, the bacteriological status of the patient and upon the occurance of the adverse events. If a regimen provides three effective drugs from groups 1, 2 and 4 (table 2) in addition to the injectable, the latter drug could probably be safely withdrawn when the smear and/or culture becomes negative. A surrogate of the effectiveness of these three drugs (especially FQs) could be early smear negativisation. However, when there are less than three effective drugs in the regimen, or if any of them belong to group 5, a longer administration of the injectable agent should be considered. The CP in the treatment of MDR-TB is generally based on a single core drug (the FQ, unless there is drug resistance to all FQs) and other accompanying drugs.

Due to the complexity of the decisions, the choice of a treatment regimen for patients with MDR-TB (or patients who cannot use some drugs because of serious adverse effects) should always be decided by experienced clinicians following internationally accepted guidelines [27–29]. In several countries, "consilia" have been established to discuss treatment decisions and the clinical course of patients with MDR/XDR-TB among experienced healthcare professionals (fig. 1) [30].

New TB patients who are contact of a known MDR-TB case should initially receive the same regimen as the putative index case [31].

Patients with MDR-TB who were not exposed to an SLD in the past should be treated with a standardised drug regimen, until DST results becomes available. The regimen for these patients might include one group 2 drug (high-dose Lfx or moxifloxacin (Mfx)), one group 3 drug, ethionamide/prothionamide plus one other group 4 drug (preferably Cs). WHO recommends adding Z to the treatment of all cases of MDR-TB, irrespective of the DST result, as DST for this drug

Table 2. Rational and sequential categorisation of drugs used in the treatment of tuberculosis (TB)

Drug	Daily dosage
Group 1: first-line oral anti-TB drugs[#]	
H	5 mg·kg^{-1}
R	10 mg·kg^{-1}
E	15 mg·kg^{-1}
Z	30 mg·kg^{-1}
Group 2: FQs[¶]	
Lfx	15 mg·kg^{-1} (750–1000 mg)
Mfx	7.5–10 mg·kg^{-1} (400 mg)
Ofx	15 mg·kg^{-1}
Group 3: second-line injectable anti-TB drugs[¶]	
Am	15 mg·kg^{-1}
Cm	15 mg·kg^{-1}
Km	15 mg·kg^{-1}
Group 4: other less effective second-line anti-TB drugs[#]	
Cs/Trd	15 mg·kg^{-1}
Eto/Pro	15 mg·kg^{-1}
PAS	150 mg·kg^{-1}
Group 5: other less effective drugs or drugs with limited clinical experience[#]	
Amx/Clv	875/125 mg *b.d.*
Clr	500 mg *b.d.*
Cfz	100 mg
Meropenem/Clv	500–1000 mg *t.i.d.*
hdH	10–15 mg·kg^{-1}
Lzd	600 mg
Thz	150 mg

H: isoniazid; R: rifampicin; E: ethambutol; Z: pyrazinamide; FQ: fluoroquinolone; Lfx: levofloxacin; Mfx: moxifloxacin; Ofx: ofloxacin; Am: amikacin; Cm: capreomycin; Km: kanamycin; Cs: cycloserine; Trd: terizidone; Eto: ethionamide; Pro: prothionamide; PAS: para-aminosalicylic acid; Amx: amoxicillin; Clv: clavulanate; Clr: clarithromycin; Cfz: clofazimine; hdH: high-dose isoniazid; Lzd: linezolid; Thz: thioacetazone. [#]: use all possible drugs; [¶]: use only one, since they share genetic targets. Reproduced and modified from [27] with permission from the publisher.

is not reliable and often not even performed [20, 23, 28, 29]. This general recommendation may not be appropriate if the results of phenotypic and genotypic DST for Z suggest drug resistance.

The possibility of shortening the total duration of the MDR-TB regimen is suggested by a recent publication from Bangladesh, where a regimen of just 9 months (shortened) with SLDs achieved relapse-free cure rates approaching more than 89% [6]. However, only patients previously treated with FLDs were included in this study.

Finally, the MDR-TB cases having received FLDs and SLDs in the past must be managed with individualised regimens, following the recommendations proposed in this article.

Individualised treatment should always be offered to patients with XDR-TB [20, 27, 28].

It is very important to highlight that all TB patients, even those with a very extensive pattern of *M. tuberculosis* drug resistance, have a chance of being cured [21]. A positive treatment outcome depends on the access to SLDs (including those in WHO group 5; table 1) and expertise in the clinical management [21].

Surgery in the treatment of TB and MDR-TB

Surgical resection of infected lung tissue may be indicated in selected patients with pulmonary TB.

In EPTB, surgical interventions may be necessary, such as when patients suffer from constrictive pericarditis or from vertebral abscesses that are compressing the spinal cord. TB abscesses may

require surgical drainage. Inflammation related to intracranial abscesses have a tendency to increase in size during the IP and surgical interventions may become necessary if the intracranial pressure cannot be controlled by corticosteroids alone [3]. Surgical procedures may also be indicated to obtain biological specimens for microbiological and histopathological investigations.

In the great majority of patients with pulmonary TB, surgery is not indicated in view of the excellent efficacy of the pharmacological treatment. Pulmonary surgery should be considered in the treatment of patients with MDR-TB meeting the three following conditions: 1) a fairly localised lesion; 2) an adequate respiratory reserve; and 3) the lack of a sufficient number of effective drugs to design a regimen that would be potent enough to ensure the cure of the patient. Surgery should be performed by experienced surgeons with the support of efficient post-operative care units, because of the associated high perioperative morbidity and mortailty [20, 23].

New drugs in the treatment of TB

Despite the emerging problem of *M. tuberculosis* drug resistance, no new anti-TB drug has been developed and licensed for treatment during the past 45 years. In principle, a new drug for the treatment of TB should fulfil two main objectives: 1) reduction of the duration of treatment; and 2) increasing the cure rate in cases with MDR/XDR-TB.

Development of new anti-TB drugs has been complicated by the need for tremendous investments by industry. In the past, the interest of pharmaceutical companies in engaging in the development of anti-TB drugs may have been limited because the economic profit was not sufficient for a drug to be sold to patients who mainly originate from developing economies.

Today, there are more than 15 new compounds with potential anti-TB action in the pre-clinical or clinical phases of development. In addition, nearly 30 other interesting molecules have been identified in screening, lead identification or lead optimisation [32, 33].

Although FQs were not initially developed as anti-TB drugs, the later-generation FQs, gatifloxacin and Mfx, have shown excellent anti-TB efficacy and are now being formally evaluated in clinical phase III studies, with the goal of reducing the length of anti-TB treatment [33]. However, despite promising results in mice [34, 35] and humans [36], there is ongoing controversy about this possibility and, at this stage, the goal of shortening the treatment seems unlikely to be achieved with FQs [33, 37].

Drugs that are evaluated for the treatment of TB in clinical phase II are rifapentine, linezolid, bedaquiline (TMC-207), delamanid (OPC-67683) and PA-824 [33]. Based on its long half-life, rifapentine is being evaluated in a once-a-week treatment regimen [9]. Linezolid is an oxazolidine antibiotic included in WHO group 5 of drugs for the treatment of patients with extended *M. tuberculosis*-drug resistance patterns (table 2) [20, 38]. Besides very high cost, linezolid has a substantial toxicity profile when used for long periods of time [39, 40]. However, the price is a market problem and the toxicity could be reduced with aggressive management [41]. Although the clinical efficacy of linezolid for the treatment of MDR-TB is still uncertain, it seems one of the best group 5 drugs to treat these patients, especially those with XDR-TB.

Of the new compounds, delamanid and PA-824 are two nitroimidazoles giving encouraging results in the trials that have been performed to date [42–44]. Delamanid is the most promising, with a good early bactericidal activity in the phase I studies [42] and demonstrated efficacy as an add-on to a MDR-TB backbone treatment regimen in one recently published randomised placebo controlled phase IIb study [45]. Another very promising compound in clinical phase IIb is the diarylquinoline bedaquiline. Bedaquiline inhibits the proton pump adenosine triphosphate (ATP) synthase of *M. tuberculosis* and is active against drug-sensitive and -resistant strains of *M. tuberculosis* in pre-clinical evaluations. The published results of a phase IIb clinical trial with bedaquiline as an add-on to a MDR-TB backbone treatment regimen showed significant improvement in the rate of culture conversion by 2 months [46]. Treatment with bedaquiline also prevented the development of drug

resistance against companion drugs in the course of treatment [47]. In a murine model, the combination bedaquiline/R/Z and bedaquiline/H/Z cured TB within 2 months. Bedaquiline is synergistic with Z in the early bactericidal activity by 14 days of treatment [48].

Conclusions

With appropriate management and resources, more than 90% of patients with TB due to pan-drug sensitive strains of *M. tuberculosis* can be cured. Moreover, in spite of the fact that managing patients with drug-resistant strains of *M. tuberculosis* is more complicated, the majority of patients with MDR-TB can be cured, provided that the resources for a rapid diagnosis and an optimal treatment are provided.

However, with increasing levels of anti-TB drug resistance (*e.g.* XDR-TB), the treatment and care of patients with TB becomes more difficult and resource consuming while the outcome becomes less favourable, rarely exceeding 50–60% of patients cured.

There is limited evidence for the optimal combination of anti-TB drugs for the treatment of MDR/XDR-TB. Individual markers to guide the decision for the duration of MDR/XDR-TB treatment are missing.

This chapter presents the existing evidence behind recommendations for the treatment of patients with TB and different levels of *M. tuberculosis* drug resistance. Table 3 provides a summary of the topics discussed in this article with the most important recommendations related to the treatment of patients with MDR-TB.

Table 3. Multidrug-resistant tuberculosis management: fundamental aspects

Steps	Considerations
1) Diagnose	Consult information from: history of drugs (1 month of monotherapy or single drug intake over a failed regimen could be a strong predictor of resistance) DST (most reliable for R and H; also reliable for Km and FQs; less reliable for E and Z; very low reliability for group 4 drugs) Perform HIV test
2) Number of drugs	At least four effective drugs: never used in the past or susceptible according to DST, taking into account DST reliability and cross-resistance
3) Drug selection	Use FLDs if still effective One injectable SLD One new-generation FQ Use two group 4 drugs until four effective drugs are complete; the first choice must always be Eto/Pro If necessary, use group 5 drugs to strengthen the regimen or when no four effective drugs can be reached with the previous groups; count two group 5 drugs as one effective drug
4) Duration of the injectable drug	At least 4 months after smear or culture conversion Longer if there are no three effective drugs during CP or are from group 5 Generally recommended for 7–8 months
5) Surgery	Consider only if: few effective drugs are available localised lesions sufficient respiratory reserve
6) Ideal regimen	Standardised (if there is no use of SLDs in the past) Individualised (use of SLDs in the past)

DST: drug-susceptibility testing; R: rifampicin; H: isoniazid; Km: kanamycin; FQ: fluoroquinolone; E: ethambutol; Z: pyrazinamide; FLD: first-line drug; SLD: second-line drug; Eto: ethionamide; Pro: prothionamide; CP: continuation phase. Reproduced and modified from [49] with permission from the publisher.

Statement of Interest

C. Lange has participated in immunodiagnostic studies where test kits have been provided free of charge by the companies Cellestis and/or Oxford Immunotec.

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Chapter 13

Management of adverse drug events in TB therapy

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SUMMARY: Adverse drug events are encountered frequently during treatment of tuberculosis (TB). Current recommendations for their management are primarily based on clinical experiences rather than systematic clinical trials. A pragmatic approach is generally adopted, usually including pre-treatment evaluation, monitoring, treatment interruption or symptomatic management, and careful re-introduction of appropriate treatment. Proper pre-treatment evaluation, notably of liver and renal function, other drugs and psychiatric disorders, with the appropriate choice of regimens and suitable dosing, minimises the risk of drug toxicities and interactions. Patient education and close clinical or laboratory monitoring facilitate early recognition. It is desirable to avoid unnecessary drug interruption, but timely removal of the offending drug causing a major reaction is crucial for patient safety. After a drug reaction subsides, careful re-establishment of an effective treatment within a reasonably short period is essential to avoid treatment failure and drug resistance. Optimal balance of benefits and risks is required, especially when treatment options are limited by drug resistance or unfavourable clinical and social factors.

KEYWORDS: Adverse reaction, anti-tuberculosis drugs, drug interaction, management

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Despite the effectiveness of the modern 6-month, short-course combination regimen in the treatment of tuberculosis (TB), adverse drug events are encountered frequently [1, 2]. Most of these adverse events are mild and self-limiting, but some are severe or even life-threatening. Not only may adverse drug reactions disrupt treatment, patients experiencing such reactions are also more likely to default, with consequential risks of failure, relapse and drug resistance [3–5]. With the global emergence of drug resistance [6], there is an increasing need to utilise second-line drugs to treat drug-resistant (DR)-TB. These second-line drugs are more commonly associated with adverse reactions than first-line drugs [7–9]. With the HIV co-epidemic [10, 11], as well as the changing clinical profiles of our patients [12], careful management is called for, both for optimal care of the individual patient and the success of the TB control programme.

Adverse events associated with anti-TB drugs

Adverse reactions to anti-TB drugs can be either dose-dependent or idiosyncratic [13–17]. Dose-dependent adverse effects are more likely to occur in renal and hepatic impairment or drug–drug interaction, where the pharmacokinetics of a drug may be significantly altered. Idiosyncratic reactions are difficult to predict, but their occurrence or consequence may also be affected by the patient's background condition and additive toxicities incurred by concomitant medications. Clinical vigilance is required, both to avoid and recognise these adverse events, especially in the presence of other underlying medical conditions and/or drug–drug interactions.

Prevalence

The important adverse reactions to anti-TB drugs are summarised in table 1 [13–16]. The most common reactions associated with first-line drugs are gastrointestinal and cutaneous in nature [1, 2, 17–21]. Hepatotoxicity, though less common, is another concern because it has been associated with the three most important first-line drugs, namely isoniazid, rifampicin and pyrazinamide [1, 2, 17, 22–27]. Although 25–60% of patients receiving first-line drugs in some studies reported at least one type of reaction [18–20], most of these were mild and either self-limiting or responded to simple supportive treatment. Treatment interruption for 1 week or longer was required in only about 10% of patients, and about 8% of patients had one or more drug terminated (more frequently streptomycin and pyrazinamide).

Even higher frequencies of adverse events are observed with second-line drugs [7–9]. Similarly to first-line drugs, gastrointestinal adverse effects are most common but cutaneous reactions might be less frequent. Among the second-line drugs, ethionamide/prothionamide (thionamides) and para-aminosalicylic acid (PAS) can also be hepatotoxic. Hepatitis occurs rarely with fluoroquinolones [14, 28, 29]. Neuropsychiatric reactions are common with cycloserine but they also occur occasionally with the thionamides, isoniazid and fluoroquinolones [14]. Gastrointestinal, haematological and neurological reactions are commonly associated with linezolid, especially when used in twice daily doses of 600 mg or on a long-term basis [30]. Fortunately, with proper management, these adverse events led to complete cessation of drug treatment in only 2% of the patients in the first five Green Light Committee-approved projects, even though 30% required removal of the suspected drug(s) from the regimen due to adverse events [7]. Similarly, in a Hong Kong (China) study, about 40% of patients with multidrug-resistant (MDR)-TB experienced adverse drug reactions of varying severity, but only half of them required modification of their drug regimens [31]. In another study in Peru, adverse drug reactions never resulted in discontinuation of anti-TB therapy, and only occasionally (11.7%) resulted in suspension of an agent [32]. Although adverse effects of the second-line agents led to treatment modification in 55.5% of patients in a Turkish study, the treatment success rate (77.6%) did not appear to be markedly compromised, with timely and appropriate management [8]. Similar experiences were also reported in Tomsk (Russia) [33] and the aforementioned Green Light Committee-approved projects in resource-limited settings [34].

Risk factors

The frequency of adverse drug events varies not only with different drugs or combinations, but also with various host factors [13–15, 26, 35]. Table 2 summarises some of the risk factors associated with adverse drugs events during the treatment of TB. Ethnic differences in the prevalence of adverse effects are observed, and racial variations in pharmacogenomics affecting drug metabolism and response may underlie some of these differences [36–38].

Population ageing is affecting many developed and developing countries. In low- and intermediate-burden countries, increasing proportions of patients are elderly [12]. Apart from age-associated changes in drug metabolism and excretion [39], their higher incidences of comorbidities [40] may also predispose them to various adverse drug events, including interactive toxicities of the multiple drugs they may be taking.

The interaction between the HIV and TB epidemics complicates the management of either condition [10, 11]. The risk of adverse reactions increases with the degree of host immunosuppression [14, 35, 41]. Early initiation of antiretroviral therapy (ART) improves outcome and reduces mortality in TB patients co-infected with HIV [41–44]. However, there are a large number of tablets to ingest, which could result in overlapping adverse effects [14, 45–47], drug–drug interactions [14, 45–47] and immune reconstitution inflammatory syndrome (IRIS) [48–50], and potentially discourage adherence to treatment.

Other risk factors will be discussed further in subsequent sections on specific adverse events.

Clinically significant drug interactions

During drug treatment of TB, both pharmacokinetic and pharmacodynamic interactions [14, 45–47] can be encountered, but the former are more frequent, especially with rifamycins [39, 51, 52]. Table 3 lists some common drug interactions involving rifamycins [39, 51, 52], and table 4 lists some examples of potential additive toxicity between anti-TB drugs and ART. Older patients, HIV-infected subjects and recipients of organ transplants are especially at risk of drug interactions [51–53].

In humans, the cytochrome (CY)P1, CYP2 and CYP3 families of the CYP450 metabolic pathways affect the vast majority of drug metabolism [39, 54]. The currently available rifamycins are all inducers of the CYP3A isoform enzymes, with rifampicin having greater activity than rifabutin [51, 52]. Some drug interactions may also occur through modulation of both CYP450 systems and the P-glycoprotein, a 170-kDa phosphorylated and glycosylated plasma membrane protein belonging to the adenosine triphosphate (ATP)-binding cassette superfamily of transport proteins [51, 55, 56]. Rifampicin has been shown to decrease serum concentrations of protease inhibitors by 35–92%, whereas rifabutin decreases them by only 15–45% [52]. The nucleoside reverse transcriptase inhibitors, such as zidovudine and lamivudine, are not metabolised by the CYP450 enzymes. The plasma concentration of zidovudine, which is metabolised mainly by glucuronidation [57], is decreased when it is co-administered with rifampicin [58]. However, lowering of the plasma concentration has not been shown to reduce the concentration of the intracellular phosphorylated active form [57].

Many of the clinically significant interactions between isoniazid and other agents (drugs or food) are pharmacokinetic in nature, principally involving inhibition of CYP450 enzyme systems (especially CYP2E1) by isoniazid [51]. Monoamine oxidase or histaminase can also be affected. Some important examples include carbamazepine and ethosuximide, theophylline, paracetamol, diazepam, warfarin, and tyramine/histamine-containing food, especially aged or fermented products, where toxicity due to apparent overdosing of the respective agents may occasionally occur.

The interactions between fluoroquinolones and other drugs/dietary constituents pertain to altered absorption, metabolism and renal excretion of the fluoroquinolones or the co-administered agents [51]. The CYP450 system might be involved. The known pharmacokinetic interactions principally include metal cations (in antacids, anti-ulcer preparations, buffered didanosine formulations, nutrient supplement and vitamins), theophylline and phenytoin. Pharmacodynamic interactions with nonsteroidal anti-inflammatory drugs and possibly warfarin, cyclosporine and cycloserine can also occur [51]. The thionamides are thought to be metabolised by some members of the CYP450 system, but whether their doses and/or those of certain antiretroviral drugs used concomitantly should be modified is still unknown [47, 59]. Clarithromycin is a substrate and inhibitor of CYP3A and has multiple drug interactions with protease inhibitors and non-nucleoside reverse transcriptase inhibitors [14]. However, given its weak activity against *Mycobacterium tuberculosis*, clarithromycin is generally not an anti-TB drug of choice in patients co-infected with TB and HIV [14].

General principles of management

Balancing treatment need and patient safety

As untreated TB carries a high mortality as well as a public health hazard, it is desirable to avoid unwarranted drug interruption or suboptimal treatment, which may lead to treatment failure and/or

Table 1. Adverse reactions to anti-tuberculosis drugs

Drug	Reactions		
	Common	**Uncommon**	**Rare**
First-line drugs			
Isoniazid		Hepatitis Cutaneous hypersensitivity Peripheral neuropathy	Dizziness Seizure Optic neuritis Encephalopathy Haemolytic anaemia Aplastic anaemia Lupoid reactions Arthralgia Gynaecomastia
Rifampicin		Hepatitis Cutaneous hypersensitivity Gastrointestinal reactions Thrombocytopenic purpura Febrile reaction Influenza-like syndrome	Shortness of breath Shock Haemolytic anaemia Acute renal failure
Pyrazinamide	Nausea Flushing Photosensitisation	Hepatitis Vomiting Arthralgia Cutaneous reactions	Sideroblastic anaemia Gout
Ethambutol		Retrobulbar neuritis Arthralgia	Hepatitis Cutaneous reactions Peripheral neuropathy
Streptomycin	Cutaneous hypersensitivity Dizziness Numbness Tinnitus	Vertigo Ataxia Deafness Deranged renal function tests	Renal damage Aplastic anaemia
Second-line drugs			
Amikacin/kanamycin/ capreomycin	Ototoxicity: hearing damage, vestibular disturbance Nephrotoxicity: deranged renal function tests Electrolyte disturbances	Clinical renal failure	
Ofloxacin/levofloxacin[#]/ moxifloxacin[¶]	Gastrointestinal reactions Insomnia	Anxiety Dizziness Headache Tremor	Tendinitis Seizure Colitis Haemolysis
Ethionamide/ prothionamide	Gastrointestinal reactions	Hepatitis QT interval prolongation Cutaneous reactions Peripheral neuropathy	Seizure Depression Impotence Gynaecomastia Postural hypotension
Cycloserine	Dizziness Headache Depression Memory loss	Psychosis Seizure	Sideroblastic anaemia
PAS	Gastrointestinal reactions	Hepatitis Drug fever	Hypothyroidism Haematological disorders Metabolic acidosis Sodium overload
Clofazimine[+]	Photosensitisation Hyperpigmentation Cutaneous reactions	Gastrointestinal reactions Retinopathy	Intestinal obstruction

Table 1. Continued.

Drug	Reactions		
	Common	Uncommon	Rare
Amoxicillin–clavulanate[+]	Gastrointestinal reactions Cutaneous hypersensitivity	Headache	Haematological reactions Vasculitis Hepatitis Colitis Stevens–Johnson syndrome Seizure
Linezolid[+,§]	Diarrhoea Dyspepsia Peripheral neuropathy	Thrombocytopenia Aplastic anaemia	Colitis Optic neuropathy

PAS: para-aminosalicylic acid. [#]: better tolerated than ofloxacin; [¶]: carries the greatest risk of QT prolongation; [+]: may have a role in treatment of extensively drug-resistant tuberculosis; [§]: toxic effects appear common with higher doses and long-term use.

emergence of resistance. However, timely removal of the offending drug in the presence of an adverse drug reaction is crucial for patient safety. Few randomised controlled trials have specifically examined the management of adverse events occurring in the treatment of TB. However, clinical experiences with the management of adverse effects occurring during the early clinical trials [60] and extensive field use of these drugs in service settings has provided important lessons on this area. A series of reviews or guidelines have helped to summarise such experiences into specific recommendations [13–15, 17, 28, 35, 61–63]. In most of these guidelines, a careful balance is struck between the two conflicting goals: treatment need and patient safety. A pragmatic approach is generally adopted, usually including: 1) pre-treatment evaluation; 2) monitoring; 3) treatment

Table 2. Risk factors for adverse drug events complicating anti-tuberculosis (TB) treatment

Risk factor	Possible reasons
Ageing	Changes in drug metabolism and excretion Comorbidities Drug interactions
Malnutrition	Fatty liver reduces hepatocyte glutathione and, hence, neutralisation of toxic metabolites originating from drug acetylation Hypoalbuminaemia increases the unbound drug fraction
Pregnancy	Fatty liver Hypoalbuminaemia Adverse effects on fetus (*e.g.* aminoglycoside impairs hearing of newborns, quinolones cause growth cartilage abnormalities in animals, ethionamide is potentially teratogenic)
Liver or kidney dysfunction	Anti-TB drugs can cause liver or kidney toxicity Chronic hepatitis status (notably hepatitis B or C) and chronic liver diseases (notably alcoholic liver diseases) predispose to drug-induced hepatitis
Treatment with other drugs	Drug interactions, especially pharmacokinetic interaction involving CYP450
Disseminated or advanced TB	Probably a consequence of malnutrition or liver deterioration
Previous anti-TB treatment	Increased risk of hypersensitivity reactions History of adverse events may recur
Atopy	Linked to a family history of adverse anti-TB drug reactions
HIV infection	The risk of adverse reactions increases with the degree of host immunosuppression Drug interactions, both pharmacokinetic and pharmacodynamic

CY: cytochrome.

Table 3. Common drug interactions involving rifamycins

Major categories	Classified examples[#]
Drugs with concentrations reduced by rifampicin	Nine subgroups with ''anti-'' as prefix Anticoagulants: warfarin Anticonvulsants: phenytoin, lamotrigine Antibiotics: erythromycin, clarithromycin, doxycycline Antifungals: azoles (*e.g.* itraconazole, voriconazole) Antimalarials: atovaquone, mefloquine Antidepressants: nortriptyline Antiarrhythmic agents: quinidine, tocainide, propafenone Antihypertensive agents ACEIs and AII-RAs (*e.g.* enalapril, losartan) β-blockers (*e.g.* propranolol, metoprolol) Calcium channel blockers (*e.g.* nifedipine, diltiazem, verapamil) Antiretroviral agents Protease inhibitors (*e.g.* indinavir) NNRTIs: delavirdine Anxiolytics: diazepam Bronchodilators: theophylline Cardiac glycosides: digitoxin Hormonal agents: combined and progestogen-only pills, tamoxifen, levothyroxine Hypoglycaemics (sulfonylurea): tolbutamide, chlorpropamide Immunosuppressants: corticosteroids, cyclosporine, tacrolimus, mycophenolate Lipid-lowering drugs: simvastatin, fluvastatin Narcotics: methadone
Drugs that increase rifabutin concentrations	Antibiotics: clarithromycin Antifungals: fluconazole Protease inhibitors: ritonavir
Drugs that reduce rifabutin concentrations	NNRTIs: efavirenz

ACEI: angiotensin-converting enzyme inhibitor; AII-RA: angiotensin II receptor antagonist; NNRTI: non-nucleoside reverse transcriptase inhibitor. [#]: list not inclusive.

interruption or symptomatic/supportive management; and 4) careful reintroduction of appropriate treatment. Difficult decisions have to be made in situations where treatment options are limited by drug resistance, especially in the presence of unfavourable clinical or social factors in resource-limited settings [14]. Active involvement of the patient in the decision-making process is necessary, and psychosocial support is often critical for the patient to overcome the discomfort, fear and anxiety throughout the whole process [10, 14].

Many of the above recommendations have stood the test of time, as reflected by the generally favourable treatment outcome of both drug-susceptible TB [18–20] and DR-TB [8, 33, 34], despite the frequent occurrence of these events. Although second-line drugs have many more adverse effects than first-line anti-TB drugs [7–9], experience has shown that effective management of these adverse events is possible even in resource-poor settings [7, 32].

Pre-treatment evaluation

With the relatively frequent occurrence of adverse events in the treatment of TB, proper clinical assessment to identify risk factors for adverse drug reactions is important before starting anti-TB treatment [13, 15]. For treatment with first-line drugs, the World Health Organization (WHO) advocates a symptom-based approach and does not recommend pre-treatment laboratory screening in absence of clinical indications [13]. However, where resources are available, baseline blood tests may help to identify risk factors and provide a basis for comparison in case of problems. The American Thoracic Society (ATS) recommends measurement of aspartate transaminase (AST)/

Table 4. Examples of potential additive toxicities between antiretroviral therapy (ART) and anti-tuberculosis (TB) drugs

Toxicity	Antiretroviral agent	Anti-TB agent
Peripheral neuropathy	*Stavudine, didanosine, zalcitabine*	*Linezolid, cycloserine, isoniazid,* aminoglycosides, thionamides
CNS toxicity and depression	*Efavirenz*	Cycloserine, isoniazid, thionamides, fluoroquinolones
Headache	*Zidovudine, efavirenz*	*Cycloserine*
Nausea and vomiting	*Ritonavir, stavudine*, nevirapine, most others	*Thionamides, PAS,* rifampicin, isoniazid, ethambutol, pyrazinamide, others
Abdominal pain	*All ART*	*Clofazimine, thionamides, PAS*
Pancreatitis	*Stavudine, didanosine, zalcitabine*	*Linezolid*
Diarrhoea	*All protease inhibitors, didanosine (buffered formula)*	*Thionamides, PAS,* fluoroquinolones
Hepatotoxicity	*Nevirapine, efavirenz, all protease inhibitors* (ritonavir > other protease inhibitors), *all NRTIs*	*Pyrazinamide, isoniazid, rifampicin,* PAS, thionamides
Skin rash	*Abacavir, nevirapine, efavirenz*, *stavudine*, others	*Streptomycin, PAS, ethambutol*, others
Lactic acidosis	*Stavudine, didanosine, zidovudine, lamivudine*	*Linezolid*
Renal toxicity and electrolyte disturbance	Tenofovir (rare)	*Aminoglycosides, capreomycin*
Bone marrow suppression	Zidovudine	*Linezolid*, rifampicin, rifabutin, isoniazid
Optic neuritis	Didanosine	*Ethambutol*, thionamides, isoniazid
Hyperlipidaemia	*Protease inhibitors, efavirenz*	None
Lipodystrophy	*NRTIs* (especially stavudine and didanosine)	None
Disturbed blood sugar regulation	*Protease inhibitors*	*Gatifloxacin*, thionamides
Hypothyroidism	Stavudine	*Thionamides, PAS*

Commonly incriminated drugs are in italics. CNS: central nervous system; PAS: para-aminosalicylic acid; NRTI: nucleoside reverse transcriptase inhibitor. Reproduced and modified from [14] with permission from the publisher.

alanine transaminase (ALT), bilirubin, alkaline phosphatase, serum creatinine and blood count in all adults before the start of anti-TB treatment [15, 26]. With the more frequent adverse events associated with second-line drugs, relevant laboratory evaluations should also be performed in the management of DR-TB, especially regarding renal and hepatic dysfunction, metabolic disturbances and haematological toxicity [13, 14]. With the use of ethambutol, testing of visual acuity (Snellen chart) and colour vision (Ishihara tests) should also be performed [15]. HIV testing should be offered after obtaining informed consent [13, 14].

Although risk factors are almost never a contraindication for treatment of either drug-susceptible TB or DR-TB, modifications of treatment regimens may be necessary to minimise the risk of adverse reactions [13–15]. In particular, dose-related adverse events are preventable by proper dosing of the medications [64]. Suitable dose adjustment or regimen modification may also be necessary in renal insufficiency and chronic liver diseases (see later sections on nephrotoxicity and hepatotoxicity), especially for drugs eliminated or metabolised by these routes [13–15]. Most drugs should be started at full dose, except for cycloserine, ethionamide and PAS, where the dose may be increased over 2 weeks [14, 63]. The patient should be advised to avoid alcohol, and both physicians and patients must be alert to the possibility of drug interactions [51–54]. Patients taking pyrazinamide, clofazimine or fluoroquinolones should be warned to limit sun exposure and to use sunscreens to minimise the risk of phototoxicity [63].

Females of childbearing age should be asked about current or planned pregnancy before starting TB treatment. With the exception of streptomycin, the first-line anti-TB drugs are safe for use in pregnancy [13]. Female patients of reproductive age should be questioned specifically about the type of contraception, if any, they are using. Females using any kind of hormonal contraceptives should be warned that rifampicin may reduce the efficacy of the pills, and they should switch to another method of contraception or, following consultation with a clinician, an oral contraceptive pill containing a higher oestrogen dose (50 μg).

A pregnancy test should be performed during the initial evaluation of females of reproductive age before treatment for DR-TB [10]. Birth control is strongly recommended for all nonpregnant females receiving therapy with second-line drugs because of potential adverse reactions in both mother and fetus. While pregnancy is not a contraindication for treatment of DR-TB, the risks and benefits should be carefully considered in the treatment approach, taking into consideration gestational age and disease severity [14]. If the patient's condition permits, the use of potentially teratogenic drugs should be avoided in the first trimester [14]. Ethionamide should be avoided, if possible, as it can increase the risk of nausea and vomiting associated with pregnancy, and teratogenic effects have been observed in animal studies [63]. If feasible, injectable agents should be avoided at least in the first 20 weeks of pregnancy because of ototoxicity. If this is not possible, capreomycin would be the preferred injectable drug [14, 63].

Pyridoxine supplementation at 10–25 mg per day is recommended for all pregnant or breastfeeding females taking isoniazid, and in similarly treated patients with HIV infection, alcohol dependency, malnutrition, diabetes, chronic liver disease or renal failure [9, 15]. Pyridoxine prophylaxis is also recommended for all patients receiving cycloserine, ethionamide or linezolid to prevent neurological adverse effects [63]. For patients receiving cycloserine or terizidone, the recommended dose is 50 mg for every 250 mg of cycloserine or terizidone prescribed [14, 63].

The recommended first-line ART regimens for TB patients are those containing efavirenz, since the interaction of efavirenz with anti-TB drugs is minimal [47]. If an ART regimen containing a boosted protease inhibitor is needed, it is recommended to give a rifabutin-based regimen [14, 47]. However, being a partial substrate for CYP3A, rifabutin may accumulate and predispose to uveitis in the presence of CYP3A inhibitors. If rifampicin has to be used, a boosted antiretroviral regimen containing lopinavir or saquinavir with additional ritonavir dosing is recommended, but close clinical and hepatic enzyme monitoring is required [43]. Attention must also be paid to the possibility of additive toxicities between ART and anti-TB drugs, as outlined in table 4.

Monitoring

Health personnel, patients and their relatives should be educated on potential adverse drug events to allow effective clinical monitoring [13–15, 35, 63]. Patients should be reminded to report symptoms promptly. At every encounter during directly observed therapy (DOT) and clinical follow-up, the patient should be screened for adverse effects of medication. Patients receiving ethambutol should be questioned regarding visual disturbances at monthly intervals [13, 15]. For treatment durations exceeding 2 months or ethambutol doses exceeding 15–20 $mg \cdot kg^{-1} \cdot day^{-1}$, the ATS also recommends monthly testing of visual acuity and colour vision [15].

Routine laboratory monitoring is not necessary during treatment with the standard anti-TB treatment regimen, unless there are risk factors or other clinical indications [13, 15]. Close monitoring is essential during treatment of DR-TB patients with second-line drugs, especially in presence of risk factors such as diabetes mellitus, renal insufficiency, acute or chronic liver disease, thyroid disease, mental illness, drug or alcohol dependence, HIV infection, pregnancy and lactation, and others [14, 63]. Laboratory monitoring is invaluable for detecting adverse reactions that escape clinical detection. Table 5 shows the minimal frequency of essential laboratory monitoring during the treatment of DR-TB, as recommended by WHO [14]. Therapeutic drug monitoring may also be considered in the case of potential drug interaction, or when careful titration of drug dose is required to maintain efficacy while avoiding adverse effects [39].

Table 5. Suggested laboratory monitoring during treatment of drug-resistant tuberculosis

Laboratory monitoring	Recommended frequency
Serum creatinine	Baseline, then monthly while on injectable agents
Serum potassium	Monthly while on injectable agents Every 1–3 weeks in HIV-infected patients, diabetics and other high-risk patients
TSH#	Half-yearly while on thionamides and/or PAS plus monthly clinical monitoring for symptoms of hypothyroidism
Serum liver enzymes	Monthly if at risk for or with symptoms of hepatitis Every 1–3 months if receiving pyrazinamide for extended periods
HIV screening	Baseline and repeat if clinically indicated
Pregnancy test	Baseline for females of reproductive age and repeat if indicated
Screening for opioids	On clinical suspicion, if a fluoroquinolone, especially moxifloxacin, is included in regimen
Haemoglobin and white blood cell count	Initial weekly monitoring if on linezolid, then monthly or as needed Initial monthly monitoring for HIV-infected patients on a zidovudine-containing ART regimens, then as needed
Lipase	Indicated for work-up of abdominal pain to rule out pancreatitis in patients on linezolid, stavudine, didanosine or ddC
Lactic acidosis	As required for diagnostic work-up of lactic acidosis in patients on linezolid or ART
Serum glucose	Weekly if on gatifloxacin plus health education on symptoms and signs of hypoglycaemia and hyperglycaemia

TSH: thyroid-stimulating hormone; PAS: para-aminosalicylic acid; ART: antiretroviral therapy; ddC: dideoxycytidine. #: sufficient for screening for hypothyroidism; it is not necessary to measure thyroid hormone levels. Reproduced and modified from [14] with permission from the publisher.

Treatment interruption or symptomatic treatment

Prompt evaluation of reports of discomfort or toxicity is extremely important, not only for early recognition of adverse drug effects but also for allaying anxiety and maintaining the patient's adherence to the treatment regimen. However, not all adverse events occurring in the course of treatment are drug induced. For example, fungal infection or scabies may be mistaken for drug rash, thrombocytopenia due to hypersplenism for rifampicin-induced thrombocytopenia, and senile purpura for thrombocytopenic purpura. Adverse events may also occur as a result of drug interaction (tables 3 and 4) or reconstitution of immunity, especially in HIV-infected patients. Clinical acumen with meticulous attention to the patient's past health, the time-course of the event (onset, duration, associated symptoms and temporal relationship with anti-TB drug therapy) and careful examination is often necessary to sort out what has actually happened. Where necessary, further investigations should be conducted either to confirm the diagnosis or to exclude alternative causes.

Severity of adverse events is assessed clinically and aided by blood tests as appropriate. Reference may be drawn to the relevant grading tables provided by WHO [65] or the National Institute of Allergy and Infectious Diseases [66]. In general, mild adverse events are either limited in extent or associated with mild derangement in blood tests. Regard must also be paid to the patient's baseline condition and the time-course of disease evolution. Owing to potentially risky consequences, some adverse events are always considered severe; for example, thrombocytopenic purpura, retrobulbar neuritis and convulsions.

The decision to continue or suspend treatment hinges on risk assessment. Some of the milder adverse effects may disappear or diminish with time [17, 13–15]. An attempt should be made to manage them symptomatically under close monitoring without treatment interruption, especially if alternative drug options are limited by drug resistance [14]. Dose reduction may be considered for dose-dependent adverse effects, but split doses should be avoided, with possible exception of the thionamides [14, 28]. If necessary, therapeutic drug monitoring may be performed to ensure

an adequate serum level for therapeutic efficacy. Treatment suspension is necessary for serious adverse events or when more conservative measures fail. Hospital care may also be required for immediate management of potentially life-threatening reactions. Table 6 summarises actions that may be taken for managing some of the commoner adverse drug events [13–15, 17, 28, 35, 63].

Reintroduction of drugs

Although it may be necessary to suspend drugs for up to 4 weeks in severe cases of adverse drug events, prolonged interruption of treatment may jeopardise the chance of cure, increase the risk of drug resistance or extend the overall treatment duration. Attempts should, therefore, be made to resume effective treatment as soon as adverse drug reactions have subsided. When TB is severe or if disease progresses after treatment suspension, interim treatment with alternative drugs that do not share a similar toxicity may be required, especially in the case of hepatotoxicity [13, 15, 26].

While it may be possible to ascribe relatively specific adverse events to certain drugs (table 1), it is often difficult to pinpoint the exact offending agent in the first encounter with gastrointestinal intolerance, hypersensitivity reaction or hepatitis. In such cases, it is usually necessary to reintroduce the original drugs sequentially to identify the culprit [17, 26]. Drug challenge aims to identify the responsible drug in the shortest possible time, rather than desensitising the patient to the drug concerned. Re-challenge with drugs suspected to have caused very severe or potentially life-threatening toxic reactions should be avoided if possible [13, 28, 35]. For example, re-challenge with rifampicin should be avoided in thrombocytopenic purpura, haemolytic anaemia, acute renal failure or shock suspected to be related to the drug. Slightly different approaches are adopted for hypersensitivity reactions and drug-induced hepatitis because hypersensitivity reactions usually occur within 2–3 days of rechallenge in a sensitised host while drug-induced hepatitis often takes 1 week or more to develop. Attempts should be made to establish an effective regimen within a reasonably short period. During the process, it is essential to avoid monotherapy of any significant duration (for example, over 1 week [35]). Drugs previously not used or unlikely to cause similar effects may be added if necessary.

Cutaneous reactions

Flushing

Flushing and/or itching of the skin with or without a rash may occur 2–3 hours after drug ingestion, especially with rifampicin or pyrazinamide. It usually involves the face and scalp, and there may also be redness/watering of the eyes. The reaction is usually self-limiting [17, 28]. An antihistamine may be prescribed if necessary, but it may not relieve flushing associated with pyrazinamide. If there are, in addition, palpitations, headache, and/or increased blood pressure, or hypotension occurring immediately after ingestion of tyramine- or histamine-containing foods, interaction between isoniazid and the ingested foods is likely and such food items should be avoided [17, 67].

Hypersensitivity reactions

A full range of cutaneous hypersensitivity reactions may occur with any medication, varying from itching with or without transient morbilliform rash (mild), frequent or prolonged rash with or without fever (moderate), to rash and fever plus chronic eczema involving limbs, generalised lymphadenopathy, hepatomegaly, splenomegaly, periorbital swelling, lips and mucosa of the mouth, Stevens–Johnson or Lyell syndrome, toxic epidermal necrolysis, thrombocytopenia, hepatitis, or nephritis (severe to very severe) [17, 63, 68]. Anaphylaxis does rarely occur within minutes of medication intake with classical signs of airway compromise and/or shock [63].

For mild skin reactions without obvious alternate causes, the recommended approach is to try symptomatic treatment with antihistamines and skin moisturisers, while TB treatment is

continued under close clinical monitoring [17, 63]. Some benefit may occasionally be achieved by changing the brand of the drug, if the hypersensitivity is due to some excipient of the product. For moderate and severe reactions or when mild skin reactions fail to respond to symptomatic treatment, anti-TB drugs should be stopped.

After the skin rash subsides, an attempt should be made to identify the responsible drug by rechallenging with each drug one by one every 1–3 days under close observation, preferably in the order of increasing risk of hypersensitivity, but rechallenge of a strongly suspected drug should be avoided, if possible, for severe reactions [17, 28, 68, 69]. Table 7 shows an example of drug rechallenge protocol for serial reintroduction of drugs. A starting dose around one-sixth of the full dose is recommended (in the belief that it may cause a lesser reaction) [17, 69]. A lower starting dose may be used for severe reactions. This is followed by rapid escalation to the full dose [68]. The offending drug should be removed if reaction recurs. Drugs are added sequentially until an effective regimen is established.

If a reaction occurs during drug rechallenge and the causative drug cannot be readily replaced or discontinued, drug desensitisation may be considered, except for severe skin reactions or those involving the mouth or mucous membranes [17, 63, 67]. Desensitisation is most commonly attempted with isoniazid and rifampicin because of the important roles of these two drugs in TB treatment [70–72]. Desensitisation should be carried out in a hospital or clinical area with the ability to monitor and respond to possible anaphylaxis [63]. Rapid emergence of drug resistance has been reported during desensitisation [73, 74]. To minimise this, the patient should be receiving two or more anti-TB drugs before undergoing drug desensitisation [17, 67].

Three different approaches have been adopted for desensitisation. In the conventional approach [17], one-sixth (or one-tenth) of the recommended dose is initiated on a twice-daily schedule, with a steadily increasing (or doubling) of each subsequent dose if tolerated, or falling back to the last tolerated dose if reaction occurs. After the recommended daily dose (split into two separate doses) is reached, continue twice-daily administration for 3 days before resuming daily dosing. A rapid oral desensitisation protocol for patients with penicillin allergy [75] has been used for desensitisation to isoniazid or rifampicin [70] and to rifampicin and ethambutol [71], with dose increase every 15 to 45 minutes. A graded incremental approach with drug administration six times daily has also been employed successfully [72]. Once desensitisation has been completed successfully, the patient should take medication 7 days per week for the remainder of treatment to avoid another, possibly more severe, reaction [28]. Some authorities recommend giving prednisone (1–2 $mg \cdot kg^{-1}$ body weight per day) for 3 days before desensitisation and continuing the steroids for up to 2 weeks before gradual tapering [35]. A case series reported the use of prednisone ranging 20–80 mg daily during desensitisation [76]. Giving oral corticosteroids twice daily may also control drug fever more effectively than a bigger overall dose given once daily.

Gastrointestinal reactions

Nausea and vomiting

Nausea and vomiting occur commonly with anti-TB drugs, especially PAS, clofazimine and thionamides [14, 28]. Clinical assessment in relation to past gastrointestinal problems, temporal relationship to TB drugs and associated symptoms is important [28]. Hepatotoxicity and other acute or chronic medical/surgical problems should be excluded [28, 63]. In females of reproductive age, pregnancy has to be ruled out. Symptoms may improve when drugs are taken after food and/or an antiemetic is taken [17, 28], but caution should be exercised regarding interaction with food (table 8) [35, 77] or antacids [51]. Switching to a daily regimen, fixed-dose combination drugs or alternative preparations (*e.g.* PAS granules) may also help [28]. Drugs, if self-administered, may also be taken before sleep. The administration of different drugs may be separated by a few hours, but split doses of individual drugs should be avoided, with possible exceptions for the thionamides and PAS [14, 28, 63].

Table 6. Suggestions for the management of common adverse drug events

Adverse events	Drugs likely to be involved	Recommended actions
Nausea/vomiting	*Ethionamide/prothionamide, PAS,* rifampicin, isoniazid, ethambutol, pyrazinamide, others	Exclude hepatitis with blood tests Administer drugs with some food (watch out for possible food interaction) If necessary, an antiemetic may be prescribed for relief of symptoms Avoid antacids if possible because they may reduce absorption of isoniazid, rifampicin and fluoroquinolones Consider daily regimens if symptoms are related to the bulk of medications in intermittent regimens Fixed-dose combination tablets may help Avoid split doses as they may result in suboptimal drug levels Medication at bedtime may help if treatment is self-administered
Nonpetechial rash	*Streptomycin, PAS, rifampicin, ethambutol,* others	Exclude other causes Mild cases: symptomatic relief with antihistamines and/or topical steroid; watch out for progression and/or mucosal involvement Moderate-to-severe rash: suspend treatment; give antihistamines; prescribe systemic steroids and other life-supporting treatment as required in life-threatening situations After rash subsides, sequentially reintroduce drugs to identify the offending drug with caution[#] Drugs strongly suspected of causing severe reaction should be avoided
Influenza-like syndrome	*Rifampicin* (intermittent)	Exclude hypersensitivity reactions and alternate causes Reduce dose size or change from intermittent to daily rifampicin administration
Haematological toxicity	*Linezolid*, rifampicin, rifabutin, isoniazid, others	For major haematological toxicities, identify and exclude offending drug promptly For milder and dose-dependent effects, dose reduction under close monitoring may occasionally be tried after carefully balancing benefits and risks
Arthralgias	*Pyrazinamide,* fluoroquinolones	In absence of acute joint inflammation/swelling (gout, infection, *etc.*) or tendonitis (fluoroquinolones), try symptomatic treatment; exclude other features of hypersensitivity If symptoms persist, consider referral for rheumatological evaluation Check serum uric acid level and consider joint aspiration for acute joint inflammation and swelling For gout associated with pyrazinamide and, rarely, ethambutol, treat with NSAID (indomethacin) or colchicine; for recurrent episodes with continuation of pyrazinamide or ethambutol, consider colchicine prophylaxis For significant inflammation of tendons or tendon sheaths, stop fluoroquinolones if possible; if essential, evaluate the fluoroquinolone dose and reduce if possible; rest the affected joint and avoid strenuous activity to prevent tendon rupture
Hepatotoxicity	*Pyrazinamide, isoniazid, rifampicin,* ethionamide/prothionamide, PAS	When hepatitis is suspected clinically, treatment may be withheld before blood test results are available Withhold treatment if: 1) ALT $>3\times$ULN + either bilirubin $>2\times$ULN or symptoms compatible with hepatitis; or 2) ALT $>5\times$ULN Reintroduce drugs when ALT returns to normal/baseline or, if deemed necessary, when ALT $<2\times$ULN[#] In case of treatment need, a nonhepatotoxic interim regimen (based on streptomycin, ethambutol and a fluoroquinolone) may also be employed

Table 6. Continued.

Adverse events	Drugs likely to be involved	Recommended actions
Nephrotoxicity	*Aminoglycosides, amikacin, capreomycin*	Discontinue the suspected injectable, maintain hydration and give supportive treatment as appropriate Monitor serum creatinine; check electrolytes and replace if necessary Adjust dosage of other drugs excreted by renal route as necessary according to creatinine clearance After recovery, consider dosing 2–3 times per week if an injectable is essential and tolerated; consider using capreomycin if an aminoglycoside was used previously
Peripheral neuropathy	*Cycloserine, linezolid, isoniazid,* ethionamide/prothionamide, aminoglycosides, capreomycin	Pyridoxine 100–200 mg·day^{-1} Correct nutritional deficiencies and electrolyte imbalance, if present For ethionamide, cycloserine or fluoroquinolones, reduce dose if possible Physical therapy, NSAID or paracetamol may also be helpful Tricyclic antidepressant (if not on linezolid), carbamazepine and gabapentin may be considered if necessary
Ototoxicity	*Aminoglycosides, capreomycin*	For significant hearing loss or vestibular toxicity attributable to the injectables, they should generally be stopped For mild and/or nonspecific symptoms (*e.g.* mild giddiness, fullness of ear, occasional ringing), consider switching to intermittent dosing with injectables under close monitoring with or without dose reduction if injectables are considered essential
Oculotoxicity	*Ethambutol,* ethionamide/prothionamide, isoniazid, linezolid, rifabutin (pan-uveitis), clofazimine	Withhold the offending medication, pending more thorough evaluation by an ophthalmologist If drug-induced oculotoxicity is confirmed, the offending drug should not be used again, except for rifabutin, which may often be continued with suitable dose reduction
Depression	*Cycloserine,* ethionamide/prothionamide, isoniazid	Psychosocial support should be provided for mild reactive depression Psychiatric assessment (including assessment of suicidal ideation) and antidepressant treatment should be considered with more cases Reduce dose of suspected agent if possible Discontinue suspected agent if there is alternative or if above measures fail
Psychotic symptoms	*Cycloserine, isoniazid,* fluoroquinolones, ethionamide/prothionamide	Withhold suspected agent while psychotic symptoms are brought under control Initiate antipsychotic therapy with psychiatric assessment Lower dose of suspected agent if possible Discontinue suspected agent if there is alternative or if above measures fail
Seizures	*Cycloserine*, isoniazid, fluoroquinolones	Withhold suspected agent Initiate anticonvulsant therapy (*e.g.* phenytoin, valproic acid) Increase pyridoxine to maximum daily dose (200 mg·day^{-1}) Reinitiate suspected agent at lower dose, if essential to regimen Discontinue suspected agent if there is alternative or if above measures fail
Hypothyroidism	*PAS, ethionamide/prothionamide*	Initiate thyroxine therapy

Italic type indicates drugs more strongly associated with the corresponding adverse event. PAS: para-aminosalicylic acid; NSAID: nonsteroidal anti-inflammatory drug; ALT: alanine aminotransferase; ULN: upper limit of normal. #: see ''Hypersensitivity reactions'' section in the main text.

Table 7. An example of a drug rechallenge protocol after subsidence of the cutaneous reaction

Sequence of reintroduction	Challenge doses mg		
	Day 1[#]	Day 2	Day 3
Isoniazid	50	300	300
Rifampicin	75	300	Full dose
Pyrazinamide	250	1000	Full dose
Ethambutol	100	400	Full dose
Streptomycin	125	500	Full dose
Levofloxacin[¶]	100	250	Full dose
PAS	500	1000	Full dose
Ethionamide/prothionamide[¶]	250	500	Full dose
Cycloserine[¶]	250	500	Full dose

PAS: para-aminosalicylic acid. [#]: for serious cutaneous reaction, the day 1 challenge dose should be reduced to one-tenth of the dose currently shown, if drug challenge is still contemplated; [¶]: relatively infrequent cutaneous reactions.

Diarrhoea

Diarrhoea usually refers to the passage of three or more loose bowel movements per day [28]. Though less frequently encountered than nausea and vomiting, diarrhoea is also commonly associated with PAS, clofazimine and thionamides, and other anti-TB drugs may also be implicated [28]. Recent unusual food/drug intake and associated symptoms should also be examined to exclude common alternative causes or other acute or chronic medical/surgical problems. Symptomatic management is recommended, with suspension of all drugs until diarrhoea resolves. Switching from intermittent to daily dosing and separating the administration of different drugs by several hours may be tried if necessary. If diarrhoea continues and an alternate regimen cannot be used, consider the addition of an antimotility agent (*e.g.* loperamide), but adsorbents should not be prescribed because of possible interference with the absorption of anti-TB drugs [28, 63]. Sequential drug challenge every 3–4 days to identify and exclude the offending agent may be resorted to if other measures fail. Investigation for *Clostridium difficile*-associated diarrhoea should also be considered for protracted or severe symptoms [78, 79].

Nephrotoxicity

Nephrotoxicity is a known complication of the injectable drugs, including both aminoglycosides and capreomycin [14, 28, 63]. The nephrotoxicity of kanamycin or amikacin is higher than that of

Table 8. Interaction between anti-tuberculosis drugs and food

Drugs best taken on an empty stomach	Drugs best taken with food
Rifampicin Absorption is reduced up to 26% by food[#]	**Rifapentine** Fatty meal enhances absorption
Isoniazid Absorption is reduced by 57% in the presence of food, particularly carbohydrates Avoid liquids containing abundant glucose or lactose Avoid food containing abundant tyramine or alcohol	**PAS** Fatty food enhances absorption Orange juice and antacids had minor effects Acidic drinks or yoghurt prevent release in stomach, reducing the incidence of nausea
Cycloserine Food reduces C_{max} by 30%, prolongs T_{max} 3.5-fold Orange juice (and probably also other acid beverages) reduces C_{max} by 15% If possible, administer with water and between meals	**Clofazimine** Fatty food increases C_{max}

C_{max}: maximal serum concentration; T_{max}: time to C_{max}; PAS: para-aminosalicylic acid. [#]: particularly fat.

streptomycin [80, 81]. Magnesium and potassium wasting may occur, especially with capreomycin [82]. Risk factors for nephrotoxicity include old age, underlying renal impairment, high blood concentration (especially trough) of injectables, prolonged injectable use, concurrent use of other nephrotoxic drugs or loop diuretics, hypotension, dehydration and liver disease [28, 83–85]. In the presence of such risk factors, pre-treatment screening and serial monitoring of serum creatinine and potassium are indicated [13–15, 28]. Estimation of the glomerular filtration rate may help to further stratify the risk in these patients. Table 9 shows the recommended dose adjustment in patients with creatinine clearance <30 mL·min^{-1} or receiving haemodialysis [14].

No change in the dosing of isoniazid and rifampicin is necessary in chronic renal failure as they are metabolised or eliminated mainly through the hepatic route. Dose adjustment, usually by decreasing the frequency of administration, is required for ethambutol, as well as pyrazinamide, as some of its metabolites are excreted through the kidneys [13, 15]. Because of an increased risk of nephrotoxicity and ototoxicity, injectables should be avoided in patients with renal impairment/failure. If their use is necessary, the dose and/or the interval between dosing should be adjusted initially as shown in table 9, with subsequent modification according to the results of therapeutic drug monitoring [13–15, 63]. The trough concentration before the next dose should be maintained below 5 μg·mL^{-1} to reduce toxicity, but a peak serum concentration less than 20 μg·mL^{-1} may not be effective [63]. For patients on haemodialysis, the medication should be given just after the dialysis session.

Nephrotoxicity of the injectables usually involves renal tubules and may present with nonoliguric acute renal failure or subacute increase in serum creatinine 1–2 weeks after treatment. The recommended management approach is as summarised in table 5. Other forms of nephrotoxicity may also occur during the treatment of TB, *e.g.* acute renal failure due to interstitial nephritis associated with rifampicin (table 1) [86].

Neurotoxicity

Peripheral neuropathy

Isoniazid, ethionamide, cycloserine, linezolid, and, rarely, fluoroquinolones and ethambutol have been associated with peripheral neuropathy, especially in patients with diabetes mellitus,

Table 9. Adjustment of anti-tuberculosis (TB) medication in renal insufficiency

Drug	Change in frequency	Recommended dose and frequency[#]
Isoniazid	No	300 mg once daily or 900 mg three times per week
Rifampicin	No	600 mg once daily or 600 mg three times per week
Pyrazinamide	Yes	25–35 mg·kg^{-1} per dose three times per week
Ethambutol	Yes	15–25 mg·kg^{-1} per dose three times per week
Ofloxacin	Yes	600–800 mg per dose three times per week
Levofloxacin	Yes	750–1000 mg per dose three times per week
Moxifloxacin	No	400 mg once daily
Cycloserine	Yes	250 mg once daily or 500 mg per dose three times per week[§]
Thionamides	No	250–500 mg per dose daily
PAS[¶]	No	4 g per dose twice daily
Aminoglycosides and capreomycin[+]	Yes	12–15 mg·kg^{-1} per dose two or three times per week

PAS: para-aminosalicylic acid. [#]: for patients with creatinine clearance <30 mL·min^{-1} or receiving haemodialysis. Standard doses are given unless there is intolerance in order to harness the potential concentration-dependent bactericidal effect of many anti-TB drugs. [¶]: use formulations of PAS that do not use the sodium salt to avoid sodium retention. [+]: caution is required in the use of injectable agents in renal insufficiency because of the increased risk of both ototoxicity and nephrotoxicity. [§]: the appropriateness of 250-mg daily doses remains to be established; monitor carefully for neurotoxicity (with or without therapeutic drug monitoring). Reproduced and modified from [14] with permission from the publisher.

alcoholism, HIV infection, hypothyroidism, pregnancy or poor nutrition, and those with inadequate dietary intake of pyridoxine [17, 28, 63]. Neuropathy associated with linezolid tends to occur after 2–4 months of therapy and may be related to both the size of the daily dose and the duration of therapy [63].

Peripheral neuropathy typically presents with prickling, tingling or burning sensation of the fingers and/or toes in a symmetric stocking glove distribution [28, 63]. This may be followed by sensory loss, absent ankle reflexes, weakness of dorsiflexion of the toes, centripetal progression with involvement of fingers and hands, and unsteadiness of gait due to proprioceptive loss. The diagnosis can usually be made clinically, but aggravating factors or alternative causes should be excluded.

Peripheral neuropathy can usually be prevented with pyridoxine prophylaxis. If symptoms develop or progress, it should be treated with pyridoxine (100–200 mg daily) [28, 63]. There are rare reports of neuropathy attributed to pyridoxine in doses exceeding 200 mg per day [85] and pyridoxine can accumulate to a toxic level in individuals with end-stage renal disease. The dose of ethionamide, cycloserine or fluoroquinolones may be reduced, but therapeutic drug monitoring may be needed to ensure an adequate drug level for efficacy [14, 63]. Physical therapy, nonsteroidal anti-inflammatory drugs (NSAIDs) or paracetamol may be helpful. A tricyclic antidepressant can be tried if disturbing symptoms persist, provided there are no contraindications (*e.g.* not on linezolid). Carbamazepine and gabapentin are other alternatives.

Ototoxicity

Aminoglycosides and capreomycin can cause both vestibular and auditory toxicity, especially with increasing age (>60 years), high serum drug concentrations, pre-existing hearing loss, coexistent ototoxins (*e.g.* loop diuretics), prior aminoglycoside use and high accumulative dose [28, 80, 87].

Vestibular toxicity may present as vertigo, incoordination, unsteadiness, dizziness, tinnitus and nausea [63], with frequency in the order of streptomycin>kanamycin≥amikacin [28, 83, 87, 88]. Transient giddiness and numbness around the mouth may occur with streptomycin treatment. Medication can usually be continued under close monitoring with dose reduction if necessary [63]. If there are early symptoms of fullness in the ears and intermittent ringing, it is sometimes possible to change the dosing to two or three times a week and continue the injectable agent for another month or more [63]. Cycloserine, fluoroquinolones, ethionamide, isoniazid or linezolid may also cause a degree of disequilibrium, and these should be carefully excluded as a cause of the symptoms [63]. Injectables should be stopped if tinnitus and unsteadiness develop, and these are attributed to vestibular toxicity [13–15, 63]. Drug-induced vestibular toxicity is generally irreversible.

Auditory toxicity presents with hearing loss, preferentially affecting the higher frequencies at the early stage [83, 89] with decreasing frequency in the order of kanamycin≥amikacin>streptomycin [28, 88, 90]. Hearing loss of 15 dB at two or more frequencies, or at least 20 dB hearing loss at one or more frequency, was found in 18% of patients treated with aminoglycosides [84]. Some degree of loss occurs in nearly all patients treated for DR-TB [14, 63]. Serial audiograms (baseline and monthly) may help to monitor patients at risk. To decrease the risk of hearing loss, consideration should be given to switching the injectables to three times per week after 3 or 4 months, with avoidance of loop diuretics and other drugs that increase eighth nerve toxicity [63]. If significant auditory toxicity develops, injectables should be stopped if other treatment alternatives are available. Hearing loss may be reversible or permanent.

Ophthalmic toxicity

Ethambutol is associated with optic neuritis, especially at doses higher than 15 mg per day and a duration of over 2 months [15, 28, 63]. Isoniazid, ethionamide, linezolid (toxic ocular neuropathy, sometimes reversible), rifabutin (reversible pan-uveitis), and clofazimine (bullseye pigmentary maculopathy and generalised retinal degeneration) are rare causes of ocular toxicity [63].

Careful pre-treatment evaluation is important for prevention. If ethambutol cannot be avoided in renal insufficiency, suitable adjustment of dose/dosing interval should be made [13, 15]. Early detection can be achieved through proper patient education, together with baseline testing and monthly monitoring of visual acuity and colour discrimination.

Optic neuritis typically presents with blurred vision, scotoma and/or red/green colour blindness [63]. Whenever a question about visual toxicity exists, the offending medication should be withheld, pending more thorough evaluation by an ophthalmologist. If drug-induced optic toxicity is confirmed, the offending drug should not be used again, except for rifabutin, where attempts may be made to reintroduce it in lower doses [63]. Nutrition deficiency, especially of the B-complex vitamins and folate, should be evaluated and corrected. Gradual improvement in vision often occurs after the offending medication is stopped, but fairly abrupt and permanent vision loss has also been reported [91].

Central nervous system toxicity

Cycloserine, thionamides, isoniazid and fluoroquinolones have been associated with central nervous system toxicity (table 6) [63].

Neuropsychiatric reactions to cycloserine are common, especially with higher doses or concomitant alcohol use [28], and these reactions may include excitement, anxiety, aggression, confusion, depression, suicidal ideation and psychosis, as well as headache, drowsiness, peripheral neuropathy, convulsions and seizures [28, 80]. Cycloserine should be avoided, if possible, in patients with a history of seizures or psychiatric problems, especially if these conditions are not under good control [14, 28]. Pyridoxine prophylaxis (50 mg for every 250 mg of cycloserine) should be given and patients advised to avoid alcohol. Patients should be cautioned about drowsiness, headache, concentration problems, irritability, mild mood changes, insomnia and agitation, which commonly occur early but typically become less problematic after the initial weeks of therapy [63]. Symptomatic treatment is recommended. Medication may be given at a time of day to minimise the effects after discussion with patient. Analgesics or NSAIDs may help headache.

Psychosocial support and psychiatric assessment should be considered for depressive symptoms [14]. An antidepressant may be tried in patients with more significant depression, although these drugs may be contraindicated when the patient is taking linezolid. Suicidal ideation should also be assessed. The dose of cycloserine and ethionamide may be reduced to 500 mg daily to see if depression is lessened. Cycloserine, and possibly ethionamide, should be stopped if the above measures fail. Isoniazid has also been associated with depression, and withdrawal of the drug is usually associated with rapid recovery [63].

For severe psychosis and seizures, the relevant acute management should be provided, all possibly offending drugs withheld and pyridoxine administered (150–200 mg) if not already given. Other possible aetiologies or contributing factors (including drug interaction) should be carefully excluded [14, 63]. After stabilisation, there should be careful clinical evaluation of the need for and risk of the relevant medications. If necessary, the drugs can be tried sequentially, preferably at a lower, but probably effective, doses under close monitoring and suitable medication cover. In case of recurrence, the offending agent should be promptly and permanently discontinued.

Hepatotoxicity

Hepatotoxicity has been associated with isoniazid, rifampicin and pyrazinamide [13–15], and less commonly with the thionamides and PAS. Of the three first-line drugs, rifampicin is least likely to cause hepatocellular damage [92], although it can be associated with cholestatic jaundice [25, 26]. Pyrazinamide is the most hepatotoxic among the three first-line drugs [1, 25, 26]. Most drug-induced hepatitis occurs within the initial 2 months of therapy [17, 24]. Deaths due to fulminant

liver necrosis have been reported, albeit rarely [93, 94]. Hepatitis occurs rarely with the fluoroquinolones [25, 29]. Most of the reported cases were rapid in onset and often had features suggestive of hypersensitivity [95].

Advanced age has been associated with increased risk of drug-induced hepatotoxicity during treatment of TB [22, 23]. Other risk factors include extensive TB disease [23, 24, 96] malnutrition [24, 96, 98], alcoholism [96, 97], chronic viral hepatitis B [97–99] and C infections [97, 100], HIV infection [99–101] and organ transplant [102–104]. Additive toxic effects may also occur between anti-TB drugs and other drugs, *e.g.* immunosuppressive drugs [102], paracetamol [105, 106] and anticonvulsants [107]. Weak genetic associations have also been reported for polymorphisms in the glutathione *S*-transferase gene [37], *CYP2E1* [37] and the *N*-acetyltransferase 2 gene (slow acetylator phenotype) [38, 96]. Patients with underlying hepatic disease or abnormality pose a significant problem in anti-TB therapy [25, 26]. Fluctuations in biochemical indicators of liver function related to the pre-existing liver problem can confound monitoring for drug-induced hepatitis [15]. Thus, drug regimens with fewer potentially hepatotoxic agents might be considered for these patients, often with omission of pyrazinamide [25]. However, chronic liver disease should be distinguished from TB involvement (often microgranuloma) of the liver, which occasionally causes abnormal baseline liver function tests that usually improve with effective anti-TB treatment [15].

Although there is some controversy regarding whether routine liver chemistry assessment should be carried out, those patients with risk factors for hepatotoxicity should have regular biochemical monitoring (table 10) [15, 61]. Drawbacks of monitoring by symptoms include nonspecificity of symptoms, and delay in diagnosis due to heightened symptomatic threshold especially among the elderly, alcoholics, substance abusers or patients with psychiatric illness [25]. A small, nonrandomised study has suggested that monitoring of liver biochemistry may help prevent severe hepatotoxicity [108]. A recent prospective cohort study showed that a risk-based approach to monitoring, as recommended by the ATS, missed 33.3% of early hepatotoxicity and 77.8% of late hepatotoxicity occurring within and after 2 weeks of treatment, respectively [109]. Abnormal liver biochemistry after 2 weeks of treatment also had a poor sensitivity of 22.2% for subsequent hepatotoxicity, irrespective of presence or absence of risk factors at baseline [109]. Further studies are therefore required to delineate the exact role of regular biochemical monitoring in the treatment of TB.

ALT is more specific than AST for hepatocellular injury [110, 111], as elevations of the latter may also indicate abnormalities in the muscle, heart or kidney. However, transient changes in bilirubin

Table 10. Suggestions on monitoring for drug-induced hepatitis in tuberculosis

Authority	Monitoring in presence of risk factors[#]	Stop drugs if clinical or symptomatic hepatitis	Cut-off levels for stopping drugs (even when asymptomatic)	
			ALT	Bilirubin
ATS [26]	Yes	Yes	5 × ULN 3 × ULN	Not specified 2 × ULN
BTS [58]	Yes	Yes	5 × ULN[¶] 2 × ULN[¶]	Not specified ↑
ERS [59]	Not specified	Yes	5 × ULN Abnormal Not specified	Not specified ↑ Jaundice

ALT: alanine transaminase; ATS: American Thoracic Society; BTS: British Thoracic Society; ERS: European Respiratory Society; ULN: upper limit of normal. [#]: especially liver diseases; [¶]: the BTS recommends weekly monitoring of liver function until normal, when ALT is two or more times normal; if the aspartate transaminase/ALT level rises to five times normal or the bilirubin level rises, rifampicin, isoniazid and pyrazinamide should be stopped.

and transaminase levels are relatively common during anti-TB chemotherapy. Up to 15% of isoniazid-treated patients develop ALT elevations even exceeding three times the upper limit of normal (ULN) but the vast majority of these are transient and asymptomatic [112, 113]. These transient ALT elevations probably represent minor liver injury, the liver "adapting" with resolution of such injury in most people [114].

Table 10 shows the cut-off levels (*i.e.* elevations in multiples of the ULN) of serum bilirubin and transaminases for withholding therapy among asymptomatic patients, as suggested by various professional authorities. The ATS recommends that TB treatment be interrupted when: 1) ALT exceeds 3 × ULN in the presence of relevant symptoms (*e.g.* anorexia, nausea, vomiting, epigastric distension, right upper abdominal discomfort, malaise and weakness) or hyperbilirubinaemia with total bilirubin exceeding 2 × ULN; or 2) ALT exceeds 5 × ULN irrespective of symptoms [26]. Notwithstanding the fact that the current treatment-limiting ALT thresholds may not accurately differentiate between hepatic adaptation from onset of significant liver injury, ALT elevations in combination with total bilirubin level elevations are well-accepted specific predictors of severe drug-induced liver injury in human clinical medicine [111, 115]. Zimmerman initially noted that patients with concurrent marked elevations in serum ALT and total bilirubin levels had at least a 10% chance of mortality from liver failure [115]. Other causes of deranged liver function, such as viral hepatitis, should be excluded [25, 26]. If AST is preferentially elevated in comparison with ALT, alcohol-related elevation of the transaminase is a possibility [26, 116]. Increase in serum total bilirubin alone can occur with a number of other causes (*e.g.* haemolysis, biliary disease, rifampicin competing with bilirubin for elimination, Gilbert-Meulengracht's syndrome or a similar condition due to concomitant use of atazanavir [28, 63]). If such a cause is found on evaluation of direct and indirect hyperbilirubinaemia, treatment may not need to be interrupted for an isolated increase in bilirubin over 2 × ULN [28].

Anti-TB drugs should be reintroduced after either full normalisation of liver chemistry [25] or when ALT is below 2 × ULN with resolution of hepatitis symptoms [26, 28, 63]. If the patient is severely ill with TB and it is considered unsafe to stop TB treatment, a nonhepatotoxic interim regimen consisting of streptomycin, ethambutol and a fluoroquinolone should be started [25, 26].

The best approach to reintroducing TB treatment is not known. In some patients, it may be possible to re-institute the "full" anti-TB regimen after recovery from drug-induced hepatitis [27, 117]. However, re-challenge with all the suspected drugs may not always be safe or necessary [25, 26, 118]. In a recent randomised control trial [117], the risk of recurrent hepatotoxicity due to simultaneous reintroduction of isoniazid, rifampicin and pyrazinamide (13.8%) was not significantly different from sequential reintroduction in full doses (10.2%) and gradually increased doses (8.6%). However, a type 2 error is likely with the small sample size, and the 13.8% risk of recurrent hepatotoxicity is by no means low, taking into account its highly unpredictable course.

Reintroducing one drug at a time may be the optimal approach, especially if the patient's hepatitis is severe. According to the ATS, rifampicin, with its lower hepatotoxicity risk [101, 119], can be introduced first, with or without ethambutol [25, 26]. After 3–7 days, isoniazid may be added if there is no increase in ALT. If ALT increases or symptoms recur, the last drug should be removed and, if necessary, the whole regimen should be re-suspended. As rifampicin and isoniazid are approximately three times less hepatotoxic in the absence of pyrazinamide [120], pyrazinamide may be withheld if previous hepatitis is severe [25, 26]. If pyrazinamide is discontinued before the patient has completed the intensive phase, the total duration of isoniazid and rifampicin therapy may be extended to 9 months [15, 25].

Alternative regimens depend on which drug is implicated as the cause of the hepatitis. If neither isoniazid nor rifampicin can be used, an attempt may be made to add other second-line drugs, especially cycloserine, to the nonhepatotoxic regimen consisting of streptomycin, ethambutol and a fluoroquinolone, with treatment continued for a total of 18–24 months [25, 26]. If rifampicin and isoniazid cannot be reintroduced together, one of them may be substituted by a fluoroquinolone,

such as levofloxacin or moxifloxacin, in the definitive TB regimen [29, 121–123] for a total duration of 1 year [25].

Aside from supportive care, no hepatoprotective agent has been convincingly shown to be useful during the hepatotoxic phase. Liver transplantation has been found to be a viable option in the treatment of fulminant liver failure resulting from severe drug-induced hepatotoxicity [124, 125]. A recent randomised trial suggested a short-term protective effect of *N*-acetylcysteine in reducing the rise of ALT and AST at 1 and 2 weeks after the start of treatment with the standard TB regimen [126]. However, with the small sample size, short follow-up and unclear outcome definition, further studies are required to confirm the findings.

Miscellaneous adverse reactions

Haematological toxicity

A diverse range of haematological reactions can follow the administration of anti-TB drugs at conventional doses [127]. Most of them are rare, with the exception of the haematological abnormalities associated with thioacetazone. Table 11 lists some of the haematological abnormalities that have been reported for various anti-TB drugs [61, 127]. Many of these reactions are idiosyncratic and/or immune-mediated. As most TB patients are given combination regimens, selective exclusion of the probable offending agent may be necessary to confirm the incriminating drug [127].

Immune-mediated thrombocytopenic purpura and haemolytic anaemia have been associated with rifamycins, especially for intermittent use at high doses [17, 28]. Dose-dependent bone marrow toxicity is very common with the prolonged use of linezolid at conventional dosage (600 mg twice daily) [30], common with attenuated dosage (600–800 mg once daily) [30] and uncommon with highly attenuated dosage (300 mg once daily) [128].

For major haematological toxicities like rifamycin-associated thrombocytopenic purpura and haemolytic anaemia, the offending drug should be stopped promptly and never be used again [17, 28, 63]. Pyridoxine-responsive sideroblastic anaemia can be prevented and treated with pyridoxine [17, 63, 127]. For isolated and asymptomatic eosinophilia, alternative preparation or batch of the same drug could be tried to exclude occasional problems associated with a particular preparation or batch. In absence of other associated hypersensitivity reactions, it is often possible to continue treatment under close monitoring. For mild linezolid-associated haematological toxicity, dose reduction under close monitoring may be tried, after carefully balancing benefits and risks [30, 128].

Influenza-like syndrome

An immune-mediated influenza-like syndrome has been associated with the use of rifamycins, especially for intermittent therapy at a high dose (>1,200 mg) [17, 28]. It occurs in up to 10% of patients receiving rifampicin 600 mg twice weekly and may also occur with daily therapy when administered irregularly (*e.g.* in noncompliant patients) [17, 28]. The syndrome usually presents after 3–6 months of intermittent therapy with fever, headache and bone pain 1–2 hours after drug administration, and generally resolves within 12 hours. Apart from symptomatic treatment, switching from intermittent therapy to daily dosing (7 days per week) will usually help to reduce its frequency and severity.

Arthralgias

Arthralgia has been associated in decreasing frequencies with pyrazinamide, fluoroquinolones, ethambutol and isoniazid [15, 17, 28]. It usually presents with mild pain and tenderness of joints (*e.g.* fingers, shoulders and knees). It usually responds to symptomatic treatment (*e.g.* NSAIDs). Uric acid level may be elevated in patients on pyrazinamide, but this, by itself, is generally not a reason to interrupt treatment.

Table 11. Haematological abnormalities associated with anti-tuberculosis drugs

Drug	WBCs	Plt	Hb	Eos	Pancytopenia	Red cell aplasia	DIC or coagulation abnormality	Haemolytic anaemia	Aplastic anaemia
Amikacin				↑					
Amox./clav.	↓	↑		↑					
Capreomycin	↓ ↑	↓		↑					
Clofazimine	↓		↓	↑			+		
Cycloserine			↓[#]						
Ethambutol	↓	↑		↑					
Ethionamide		↓							
Imipenem	↓	↓ ↑	↓	↑	+		+		
Isoniazid	↓	↓	↓[#]	↑		+	+	+	+
Kanamycin									
Levofloxacin	↓	↓	↓	↑				+	
Linezolid	↓	↓	↓[#]		+	+			
Moxifloxacin	↓	↓		↑			+	+	
PAS	↓ ↑	↓	↓[¶]	↑		+		+	+
Pyrazinamide		↓	↓[#]						
Rifabutin	↓	↓							
Rifampicin	↓	↓				+	+	+	
Streptomycin	↓	↓		↑	+		+	+	
Thioacetazone	↓	↓	↓			+		+	+

WBC: white blood cell; Plt: platelets; Hb: haemoglobin; Eos: eosinophils; DIC: disseminated intravascular coagulation; Amox./clav.: amoxicillin/clavulanate; PAS: para-aminosalicylic acid. ↓: decreased count; ↑: increased count; +: may be present. [#]: including sideroblastic anaemia; [¶]: including megaloblastic anaemia resulting from malabsorption and vitamin B12 deficiency. Data from [61, 127].

Gout may occur with pyrazinamide and, rarely, ethambutol [17, 28, 63]. If acute swelling is present, the affected joint should be aspirated to confirm acute gout and exclude infection. Indomethacin or colchicine may be used for acute attacks. Prophylactic colchicine and rheumatological evaluation may be required for recurrent episodes.

Tendonitis and tendon rupture have been reported with fluoroquinolone use, especially in older patients, patients with diabetes mellitus or chronic renal failure or those receiving corticosteroids, or when new physical activities are undertaken [63]. When significant inflammation of tendons or tendon sheaths occurs, fluoroquinolones should generally be stopped. Dose reduction with monitoring of drug level may be tried if the drug is essential. Symptomatic treatment can be given with NSAIDs with resting of the joint to avoid tendon rupture.

QT interval prolongation

The fluoroquinolones have been associated with prolongation of the QT interval, with the risk being greatest for moxifloxacin, less for levofloxacin and ofloxacin, and least for ciprofloxacin [129]. It occurs through blockade of the voltage-gated potassium channels, especially the rapid component of the delayed rectifier potassium current, expressed by the human ether-a-go-go-related gene (*HERG*) [129]. The overall risk of torsades de pointes (TdP) appears to be small [129, 130]. However, fluoroquinolones, especially moxifloxacin, should be used with caution in patients at risk for TdP. Concomitant use of other drugs causing QT interval prolongation should be avoided. QT interval prolongation has been reported for opioids, especially methadone and levomethadyl [131]. To minimise the chance of drug interaction, pre-treatment drug screening should also be considered if clinically indicated.

Hypothyroidism

Hypothyroidism may develop with either PAS or ethionamide [14, 32, 132, 133]. When both drugs are used, a high incidence of hypothyroidism has been observed (10% to over 50%) [32, 133]. Since the symptoms can be subtle, it is recommended that patients are screened for hypothyroidism by measurement of serum thyroid-stimulating hormone (TSH) at 6–9 months, and then tested again every 6 months or sooner if symptoms arise [14]. In areas where iodine-deficiency goitres are endemic, early screening may be indicated, with iodine treatment where necessary [132].

When serum TSH concentration begins to increase, clinical evidence of hypothyroidism should be sought, with more frequent monitoring of TSH. If TSH rises to 1.5–2 × ULN, thyroid hormone replacement should be instituted [63], with adjustment as necessary to return the TSH level to the normal range. The dose of thyroid hormone should be increased slowly in patients with significant cardiovascular disease. Thyroid hormone replacement can be stopped when TB treatment is complete.

Immune reconstitution inflammatory syndrome

IRIS is seen in up to one-third of TB patients started on ART in some studies but is fortunately rare in its severe forms [48–50]. This syndrome can present as a paradoxical worsening of the patient's clinical status, often due to a previously subclinical and unrecognised opportunistic infection [41, 47]. IRIS is a diagnosis of exclusion. These reactions may present as fever, enlarging lymph nodes, worsening pulmonary infiltrates, respiratory distress or exacerbation of inflammatory changes at other sites. It generally presents within 3 months of the initiation of ART and is more common with a low CD4 cell count (<50 cells·mm^{-3}) [41, 50]. A similar paradoxical response may occasionally occur with anti-TB treatment in absence of HIV infection, for example, in patients who developed TB during treatment with tumour necrosis factor (TNF) antagonists [134]. Most cases resolve without intervention. ART can be safely continued but caution is required to ensure that the ART regimen is compatible with the TB treatment [14, 41]. Various treatment modalities have been employed, including NSAIDs in mild disease and corticosteroids in moderate–severe disease.

Statement of Interest

In the past 5 years, W.W. Yew has accepted indirect sponsorship from Pfizer and GlaxoSmithKline to attend international conferences on infectious diseases and respiratory diseases.

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Chapter 14

TB in migrants

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SUMMARY: Internal and international human migration has increased worldwide in recent years. Migrants are generally people travelling from less to more economically developed geographical areas in search of jobs and better living conditions. The epidemiological profile of tuberculosis (TB) has dramatically changed in some high-income countries, partly because of migration.

In many nations, a screening system for TB disease or latent TB infection (LTBI) has been set up in order to prevent the increase of TB prevalence, dissemination of *Mycobacterium tuberculosis* strains in the local community, or new incident TB cases. Nevertheless, several reports from different developed countries with well-performing screening and treatment systems have shown in the last few years that foreign-born TB patients do not significantly contribute to *M. tuberculosis* transmission in the native population.

KEYWORDS: Latent tuberculosis infection, migrants, tuberculosis, tuberculosis screening

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Human migration can be defined as a physical movement of human beings from one geographical area to another, sometimes over long distances and/or in large groups [1]. Migrants are usually defined as foreign-born citizens who happen to live in a different country at some time in their life, irrespective of the causes, voluntary or involuntary, and the means, regular or irregular, used to migrate [1].

Driven by disasters, violence and economic disparities, internal and international human migration has increased worldwide in recent years. According to the International Organization for Migration's World Migration Report 2010 [2], the number of international migrants was estimated at 214 million people in 2010. If this estimate continues to increase at the same rate as observed during the last 20 years, it could reach 405 million by 2050.

Every year, more than 5 million people cross international borders to live in a developed country (fig. 1) [2]. Migration is economically motivated in the majority of cases, in particular, it is associated with income disparities for similar jobs in different countries. Furthermore, in some high-wage countries there is a relevant shortage of appropriately skilled or qualified citizens for some jobs.

Many temporary migrant workers travel regularly within their country or abroad. Tuberculosis (TB) is a disease associated with poverty and hardship and, because migration to and within high-income countries involves people moving from less to more economically developed areas, some countries are reporting on changes in the epidemiology of TB.

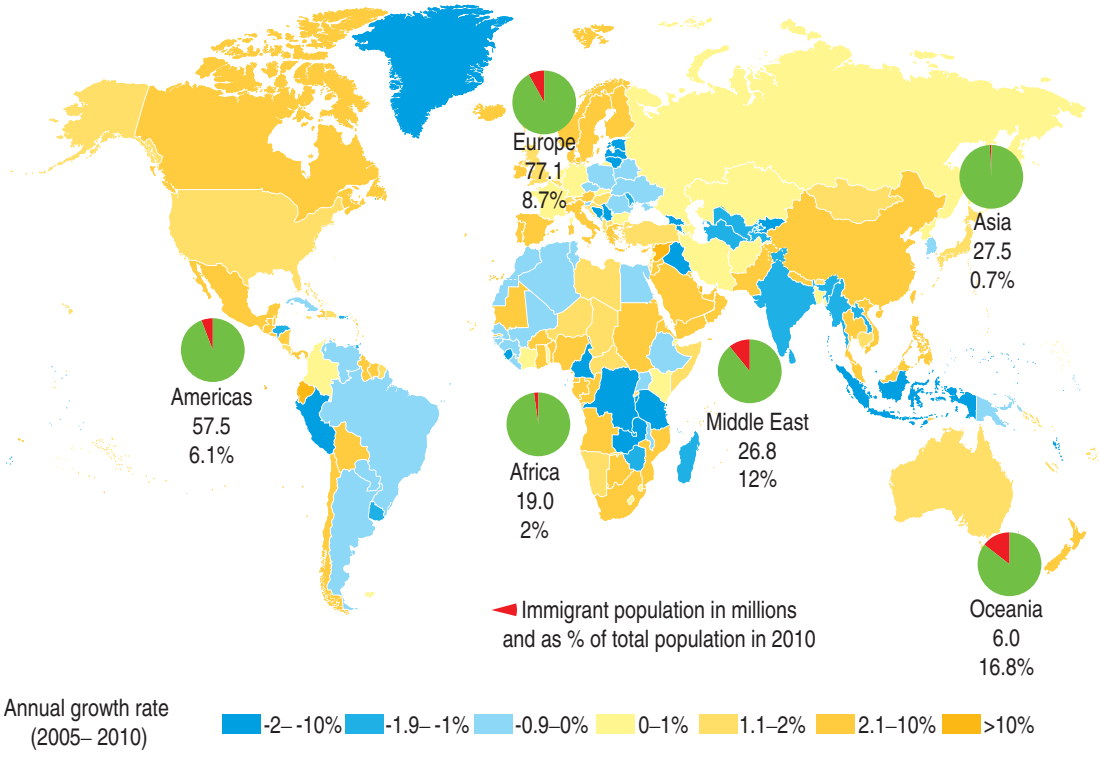

Figure 1. Immigrant growth rates (2005–2010), immigrant stocks and immigrants as a percentage of the total population in 2010. Regions in this map relate to International Organization for Migration (IOM) regions as defined in the regional overviews. Reproduced from [2] with permission from the publisher. © IOM.

The impact that immigration could have on TB control has been alerting public health authorities in developed countries for a long time [3, 4]. Many migrants originate from countries where the burden of TB disease and mortality are dramatically high. However, migrants pose a major challenge to TB control in both developed and developing countries [5].

In high-income countries located in Western Europe, North America and Australasia, which host sizeable communities of people who were born abroad, the incidence of TB has been brought down to very low levels over the last decades, and is often less than 10 TB cases per 100,000 population per year [6]. The migration process has resulted in a steady national increase of new TB cases diagnosed in immigrants and refugees living in developed countries, such as Canada and Australia, where the TB incidence of native people is low [7, 8].

The aim of this chapter is to describe the impact of migration on TB epidemiology, in particular, focusing on the European Union/European Economic Area (EU/EEA) scenario.

Epidemiology

Nowadays, the majority of TB cases detected in many high-income countries occurs in persons who are of foreign-born origin. Actually, country of birth is often used to define a case of migration-associated TB, albeit entry into the country may have occurred many years before. Many foreign-born TB patients originate from countries with a much higher TB prevalence than the country of migration, and where care and control of TB are inadequate.

While TB notification rates across Western Europe decreased by 3% yearly, overall, between 1995 and 2000, increases in notification rates were recorded in some countries, notably Denmark, Luxembourg, Norway and the UK [9], owing to an increase of TB cases in foreign-born

individuals. In 2000, TB among foreign-born persons or persons with a foreign citizenship accounted for 28% of all TB cases in Western Europe [10]. In more recent years, the overall proportion of foreign-born TB has increased from 28% in 2000 to 45% in 2010. The proportion by country, however, differs hugely and ranged from 1% in Hungary to 86% in Norway in 2010 [11]. About two-thirds of cases of foreign origin were from Asia or Africa and 20% from another European country, half of whom were from Central or Eastern Europe [12]. Similar epidemiological patterns have been observed in Canada, the USA, Japan and Australia, where foreign-born TB patients account for 60–94% of overall notified TB cases in recent years [6, 13–16].

Moreover, while there is limited evidence to suggest that those entering host countries pose a threat to host communities, it has been suggested that they may pose a threat within their migrant communities [17–19]; this could be explained by the overcrowded living conditions, which can augment the risk of exposure to susceptible individuals (children for instance) [9].

Diagnostic challenges

Medical screening related to the process of immigration commonly includes screening for TB. The rationale is to promptly diagnose and treat active, infectious TB before arrival, or as soon as possible after arrival, in order to prevent *Mycobacterium tuberculosis* transmission to persons in the host country. Secondary benefits of immigration screening are reduced transmission of mycobacterial strains in the country of origin and during travel.

Different policies recommend that diagnostic tests have to be performed before departure (*e.g.* Australia, Israel, Canada and the USA), or upon/just after arrival (*e.g.* Denmark and Switzerland). Screening methodologies and algorithms vary from country to country, ranging from clinical examination to chest radiography, the tuberculin skin test (TST) and interferon-γ release assays (IGRAs) [20–24].

TB screening strategies have shown high variability in cost-effectiveness when employed in high-income countries to test immigrants from high TB incidence countries and other individuals at increased risk of TB [25, 26].

Screening

Screening for a disease is justified if the prevalence of that disease is relatively high and if it is treatable. The ideal screening test should be inexpensive, easy to administer, cause no discomfort to the patient, and be characterised by both high sensitivity and specificity [27, 28].

Screening for TB is one of the interventions often performed to control TB among new entrants in low-incidence countries. Screening focuses on foreign-born persons who apply for immigration or who have recently arrived. The two main reasons for this approach are that: 1) TB rates are highest among recent arrivals; and 2) the application for immigration or long-term residence provides a unique opportunity for screening and is one of the few reliable points of contact with new entrants [29, 30].

In fact, screening for TB among migrant populations has been shown to lead to earlier detection of cases, resulting in a shorter duration of symptoms, fewer hospitalisations and reduced *M. tuberculosis* transmission in the community. It has been shown that screening may decrease the period of infectiousness by as much as 33% in some situations [31].

Some screening programmes are voluntary, whereas others are mandatory. Even mandatory screening is often carried out without coercion. Compliance is ensured in several ways, *i.e.* the screening result is a requirement for a residence permit, access to healthcare or social benefits, or permission to work [29, 30].

Nevertheless, a recent review showed that in the EU member states there was no indication of higher effectiveness of any of the three main strategies employed (screening at port of entry, screening just after arrival in reception/holding centres, and community post-arrival screening) [29].

Screening large numbers of migrants requires substantial economic and human resources, and it is suggested that it may be of more public health benefit to concentrate limited resources on the early detection of TB through regular services, on treatment completion and on the improvement of access to healthcare [29].

Pre-immigration screening

The objectives of a pre-departure screening are to reduce the number of contagious persons entering the country and to contain health expenses (related to diagnosis and treatment), as the legal immigrants are often granted health insurance upon arrival [21]. The major challenge in pre-departure screening is the difficulty of delivering quality-assured medical examinations and laboratory tests.

Post-immigration screening

Different approaches are employed for post-immigration screening. For example, Italian guidelines recommend an immediate screening with a chest radiograph and the Mantoux test in migrants from countries with high TB incidence after admission to Italy [32, 33]. To simplify and improve this approach and improve interaction with migrants and increase adherence, the individual's native language can be used [20].

Selecting targets for screening

Much uncertainty remains on the appropriate selection of targets for TB screening. A survey identified major differences among countries even towards those arriving for a temporary period of residence [20]. Germany, Sweden, Switzerland, the UK and the USA do not screen temporary residency applicants. Australia, Canada, France, Israel, Jordan, New Zealand, the Netherlands and Norway do screen temporary migrants who are staying longer than 3–6 months [20]. Australia uses the country of origin TB prevalence to determine which temporary migrants will be screened (*e.g.* they screen an individual if the TB rate exceeds 100 per 100,000 population and the migrant is staying >1 month) [34]. These approaches have their drawbacks if it is considered that only 2% of all foreign-born entrants to the USA seek permanent residency. In fact, students, migrant labourers, individuals visiting friends and family in the USA, other categories of visitor and persons who enter the country illegally are not screened at entry, but they may stay for many years [27].

Thus, the detection yield of active pulmonary TB may be improved through a more focused application of screening practices, either based on countries of previous residence and/or on risk factors for progression from latent TB infection (LTBI) to active disease [35–40]. On this basis, some countries address the issue of target categories selecting immigrants coming from countries with a high incidence of TB [9].

How to perform screening

Active screening allows the detection of TB at an earlier stage than passive screening that is before extensive destruction of lung parenchyma and the formation of cavities with a high bacillary load have occurred. This is expected to shorten the duration of the period of transmission of infectious particles to healthy contacts. It is, therefore, not surprising that a Swiss study reported a larger proportion of the patients still symptom-free in the actively screened group (49.3%) than in the other groups (17.6%) [41].

Chest radiography represents a relevant tool in the majority of the screening programmes. This approach has sometimes been implemented in combination with different diagnostic tools, *i.e.* stepwise symptom screening or screening for LTBI using the TST [22]. Nevertheless, based on estimates from the literature the positive predictive value (PPV) of the chest radiography is less than 1% assuming a prevalence of active disease of 1% [27].

Actually, in some settings, the focus on case finding and treatment of contacts of infectious TB cases, rather than mass screening, has been prioritised for a long time [42]. In addition, sputum examinations on asymptomatic individuals were not found to be cost-effective [42].

A recently published systematic review revealed that a mandatory approach to screening resulted in a higher coverage but not necessarily a higher TB yield [22]. This suggests that enforcing screening upon the migrant population might not have positive public health consequences. Mandatory screening possibly increases the absolute number of cases detected since the overall number screened is increased. If screening is voluntary, people who feel ill may be more likely to be enrolled than people who do not feel ill, increasing the yield [22].

Setting of screening

The setting of screening can be variable and it cannot be simply classified as pre- or post-immigration screening. The various options are presented in table 1 [22].

Pre-departure/own country

In a study from Australia, the prevalence of TB in the examined population was 137 cases per 100,000 population during the 2009–2010 financial year and 519 cases of active TB were detected. The main countries responsible for people with TB were the Philippines, India, Vietnam and China [43]. This shows that overseas screening programmes can detect substantial numbers of people with active TB who would otherwise have travelled to Australia with the disease [43].

Port of entry

Some countries, such as Switzerland and the UK, have planned to screen entering migrants at the port of entry. In Switzerland, immigrants coming from countries other than the EU/EEA member states, the USA, Canada, Australia and New Zealand who register as asylum seekers in one of the five State registration centres at the borders undergo a health check-up, which includes a TST and a chest radiograph (except for children younger than 15 years and for pregnant females), to detect any possible clinical sign of pulmonary TB [41, 44, 45].

Temporary camps or reception/holding centres

All countries with screening programmes mainly evaluate refugees and asylum seekers (known as refugee claimants in some countries) [20]. However, in Denmark, refugees in camps are only questioned about TB symptoms/screened at arrival, thus migrants only receive a very limited introduction to the Danish healthcare system [46].

In another situation described in Italy, asylum seekers and refugees are a small proportion of the total immigrant cohort, but represent a subgroup at particularly high risk of developing TB. Under

Table 1. Possible settings for screening

Setting of screening	Description
Pre-entry/pre-migration screening	Screening before arrival at the country of destination, usually carried out in the country of origin
Port of arrival screening	Screening at the airport/harbour upon arrival
Reception/holding/transit centre screening	Screening at the reception or holding centre shortly after arrival in the country (in most western countries asylum seekers are referred to special holding/reception centres to await a decision on their immigration status)
Community post-arrival screening	Screening at the community level after arrival, usually for migrants other than asylum seekers or screening of specific groups (migrant shelters or illegal migrants) in the community, *e.g.* during outbreaks
Follow-up screening	Periodic follow-up screening after the initial entry screening

Italian law, asylum seekers are housed in special reception centres. There were 44 centres in Italy in 2008, with almost 8,000 available beds [32, 47]. These reception centres are potential reservoirs in which mycobacterial strains can spread quickly, especially when there is overcrowding, increasing the risk of infection in both residents and staff. The feasibility of prophylactic and therapeutic protocols for TB in immigrants living in reception centres remains an open question [32].

Community

Many active TB cases among foreign-born individuals are attributable to the reactivation of LTBI. Reactivation rates are highest during the first 2–5 years following migration [3, 48, 49]. In fact, several reports have shown that most recent transmission among migrants was attributable to transmission from cases with the same nationality with limited transmission across ethnic subgroups [19, 46].

However, immigrant populations tend to be closed communities, which rarely lead to the initiation of outbreaks in the indigenous population. Ethnic minorities do not increase the risk of infection in the larger communities in which they settle, but do increase the risk for the small group with which they have regular contact [32, 50]. Even though transmission has been confirmed by genotype and linkage information within/between ethnic subgroups in Denmark, for example in ethnic clubs, shelters, language schools, and the environment of the homeless/socially marginalised, clustering in migrants in Denmark more likely reflects reactivation of the infection [18, 46]. In the state of Victoria (Australia), genetic typing of all new TB isolates has been performed since 1993, and, apart from limited transmission within immigrant family groups and domestic contacts (including first-generation Australian-born children), most cases of TB in migrants who have been in Australia for 10 or more years have involved unique strains [51].

In addition, restriction fragment length polymorphism (RFLP) studies have detected relatively little TB transmission from foreign-born residents to the general population [52, 53]. The estimated proportion of active TB cases among the native-born population that can be attributed to transmission from the foreign-born individuals has been reported to be as low as 2–11% [54, 55] or as high as 17% [19, 56–58]. In one US study, foreign-born TB patients were more likely to have acquired TB from USA-born individuals than *vice versa* [56]. In conclusion, in low-incidence countries the overall public health impact of TB due to foreign-born persons on the local community is modest.

Coverage of screening

Coverage of screening has been reported to range from less than 20% to almost 100% [22]. The lowest coverage was reported from a study in the port of arrival scheme in Hackney, London, UK [22]. The highest coverage, 99.8%, was reported from screening of asylum seekers in Belgium [22].

One additional strategy is to educate staff in high-risk settings such as shelters, prisons, clubs/associations for migrants and schools, to recognise and inform about TB symptoms in order to increase active case finding [46].

One key issue is loss to follow-up after the initial contact of the patient with health authorities on entry to the country. However, selective use of community language newspapers in conjunction with community health promotion initiatives as described by MILLER [59] for the Indian community in Auckland, New Zealand, are likely to be effective [59, 60].

Nevertheless, a series of studies reported extremely variable losses to follow-up ranging between 1.6% and 60.0% (median 11.5%) [22].

Contact tracing

Contact tracing generally serves different purposes, in particular [30]: 1) identifying individuals with TB disease or LTBI among the contacts of a TB patient and providing adequate treatment or follow-up; 2) reducing morbidity and mortality due to TB among newly infected individuals; and 3) reducing further *M. tuberculosis* transmission.

Some studies have already demonstrated that the risk of TB transmission to host populations from migrants is low [18, 61, 62]. For this reason, some suggest the use of contact tracing of active cases in migrants as a more efficient and cost-effective way of managing TB among the immigrant population as opposed to routine immigrant TB screening [37, 63].

One study compared contact tracing with new entrant screening in east London and concluded that contact tracing was more effective in detecting and preventing TB than new entrant screening, mainly because contact tracing selects for families or communities at particularly high risk [63]. Another research study indicated that contact tracing is highly cost-effective and can result in net savings [37].

Legal status

In different settings illegal migrants may face several challenges in the process of TB diagnosis and treatment. However, the full care and treatment of TB should be guaranteed regardless of the legal status of patients. Basic TB care should be provided free of charge.

In Australia, illegal migrants, such as Indonesian fishermen, with TB do not have the option to remain in the country to complete 6-month treatment. In fact, once they test smear negative (or culture negative in the case of multidrug-resistant (MDR)-TB), provided they have tolerated at least 1 month of anti-TB treatment, they are deported [64–66]. Thus, clinical and radiological improvement cannot be assessed, nor treatment completion confirmed.

In the Netherlands and Norway, regulations have been introduced to ensure that TB patients staying illegally in the country do not have their treatment disrupted by deportation [25]. Adapting international standards for TB care into the local policies is a key step in upholding the rights of all individuals for TB treatment regardless of origin [67, 68].

Cost

In the absence of health insurance or free care, migrants have to pay for healthcare services out of pocket, and face higher costs of care than those in their original countries [69]. Weakened family support and social networks also reduce access to care. Nevertheless, it is worthwhile to mention that, although in many countries free diagnosis and treatment are provided at government health facilities, additional costs of transport, visits to health providers and purchase of medicines can be significant for migrant patients on low wages.

Responsibility

A well-organised follow-up system is crucial for all strategies in order to maximise the yield of the entry screening system. Proper follow-up is needed in order to maximise coverage of the target group, as well as treatment adherence. After the initial medical evaluation, migrants, refugees and asylum seekers in Denmark, as in many other low-incidence countries, are covered by the national TB programme, which is based on passive case finding, treatment of active cases and contact tracing for any TB patient, irrespective of origin [70, 71]. TB care should be offered and integrated with other healthcare activities within the context of a holistic approach to ensure the health and wellbeing of new entrants.

Access

Foreign patients may face barriers to care in the host country as a result of inadequate knowledge of TB and health services, language limitations, fear of immigration authorities, unemployment and lack of money or healthcare coverage.

Migration does not necessarily pose a risk to health, but rather it is characterised by increased individual vulnerability to disease and inequalities in access to health services. The cumulative effects of deprivation (including malnutrition, underemployment and poverty), healthcare costs and psychological stress associated with the resettlement process have been cited as barriers to access to health services among migrants with TB [72].

A Chinese study showed that the majority of internal migrants had experienced significant delays between onset of illness and their TB diagnosis [73]. The delay ranged from a period of 3 months to 1 year.

Thus, improved access to care for migrants, and especially illegal migrants, is important for TB control [22, 74]. There is a relevant public health need for including high risk groups that are currently not properly covered, such as illegal migrants.

Travelling migrants

One of the greatest limitations of evaluating new immigrants and refugees is that screening is performed only once at the time of initial entry, and often only for individuals who seek permanent-resident status. In fact, there are far more foreign-born migrants entering industrialised countries under other legal statuses not requiring entry screening. In addition, permanent residents may also return to their country of origin, often doing so repeatedly [75, 76]. Two English studies estimated that 20–30% of all TB cases among foreign-born permanent residents were due to re-exposure during return visits to their countries of origin [76, 77]. Such cases will not be prevented by any screening programmes.

LTBI

The identification of migrants with LTBI provides an opportunity for the prevention of significant health sequelae [20]. This means that the screening programme must have the capacity to provide treatment for LTBI. In fact, it has been suggested that refugees, migrants and foreign-born students from high-prevalence areas should be screened for LTBI on arrival, offered treatment as appropriate and followed up beyond 10 years [51].

Diagnosis

The TST is more sensitive in detecting LTBI because chest radiographs are abnormal in only 10–20% of those with LTBI. However, the subgroup of individuals with LTBI who have abnormal chest radiographs are at an increased risk of reactivation [27]. Therefore, chest radiography screening, followed by TST screening, may be more cost-effective if this results in the treatment of fewer individuals with LTBI but who have a much higher risk of reactivation [27].

Israel, Norway and Sweden use the TST to diagnose LTBI in migrants entering the host country. While most countries do not screen for LTBI as part of the immigration screening programme, they do recommend the use of the TST to screen high risk immigrants in the primary care setting after the immigration process is completed.

The reported reasons for not using the TST [78] include: 1) a high false-positive rate due to bacille Calmette–Guérin (BCG) vaccination or to infection with environmental mycobacteria; 2) a very large number of TST-positive applicants (40–50% of adult immigration applicants have lived for 20 years or more in high TB incidence countries) [38]; and 3) low reactivation risk among many with a positive TST [46] and poor adherence to current LTBI treatment regimes in most programmes [24].

In this regard, Norway and the USA have adopted the IGRA as part of the national immigration TB screening programme. A study from Norway showed that use of IGRAs would have reduced the number of asylum seekers needing further follow up by 43% [79]. Although this would not affect the number of applicants referred for screening, use of the IGRA could improve the selection of persons to refer for further evaluation.

Treatment

Preventive therapy to certain high-risk groups, such as those co-infected with HIV, with fibrotic lesions or with recent *M. tuberculosis* infection, can reduce the pool of latently infected persons [3,

46]. Treatment with isoniazid should be administered for at least 6 months; however, implementation of a chemoprophylaxis programme in specific settings such as temporary camps or reception/holding centres can be complicated. In fact, such settings have usually high numbers of TST-positive individuals and limits of legal residence time in the reception centres can be short (sometimes not exceeding 3 months) [32, 47].

Conclusions

Several reports from different high-income countries with well-performing screening and treatment systems have shown in the last few years that foreign-born TB patients do not contribute significantly to *M. tuberculosis* transmission in the native population. They represent a significant proportion of the total TB burden of the low-prevalence countries [25, 46, 80–82].

Portrayals by the media and politicians of immigrants as the ''diseased others'' who need to be stopped at the border to avoid importation of infections to which locals are vulnerable are not only inaccurate but also ineffective [60]: inaccurate because TB in overseas-born persons rarely appears to infect the locally born; ineffective because it will miss approximately 80% of the TB in overseas-born people; and counterproductive because it makes immigrants and visitors nervous about the security of their tenure and less likely to access healthcare, and it drives their contacts underground [60].

Statement of Interest
None declared.

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Chapter 15

TB in children

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SUMMARY: Tuberculosis (TB) in childhood is under reported but represents a sentinel event of transmission in the community. Susceptibility to TB is age-dependent with young children at highest risk of disseminated disease. Age-related differences in immune responses to mycobacteria underlie this phenomenon. Since childhood TB tends to be paucibacillary, bacteriological confirmation is more difficult to achieve and accurate diagnosis remains a challenge. Diagnostics include measures of host sensitisation, such as the tuberculin skin test (TST) and interferon-γ release assay (IGRA), but their performance varies between children and adults and in the context of bacille Calmette-Guérin (BCG) vaccination. Therapeutic regimens are based on adult studies but increased doses have recently been recommended by the World Health Organization (WHO), following pharmacokinetic studies in children. TB/HIV co-infection adds complexity to diagnosis and management, much like in adults. The BCG vaccine is not fully protective and is not recommended for HIV-infected children. New vaccines are currently under investigation, with trials including infants and adolescents.

KEYWORDS: Age-related immune responses, diagnostics, epidemiology, novel vaccines, therapy, tuberculosis/HIV co-infection

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Childhood tuberculosis (TB) presents a unique spectrum of disease with its own distinct challenges for diagnosis, prevention and treatment. Herein, we highlight the major differences between adult and childhood TB.

Epidemiology

In 2010, there were 8.8 million incident cases of TB worldwide, of which 5% were in Europe. 22 high-burden countries account for 81% of all estimated cases and, overall, the TB incidence rates have fallen by 3.4% per year between 1990 and 2010 [1]. However, childhood TB remains an overlooked area within global TB control. It has been estimated that overall, children account for 10% of all TB cases worldwide, but they may account for between 15% and 25% in high-incidence countries [2]. Estimations are difficult, as until recently only smear-positive cases were reported for children under the World Health Organization (WHO) DOTS (directly observed therapy, short course) programme, yet most children present with smear-negative TB and a bacteriological confirmation is rarely sought. Notification is now based on the decision to treat for TB, and data

regarding smear-positive cases is collected separately. A recent European surveillance report identified that paediatric cases account for 4.3% of the total burden of TB in the European Union (EU)/European Economic Area [3]. Children under 5 years of age represent 48% of all paediatric cases (defined as those in children <14 years of age) and the notification rates are highest in this age group (5.42–9.21 per 100,000 in the <5 year olds *versus* 3.03–3.91 per 100,000 in the 5–14 year olds), in line with previous reports [4]. Of note, only 42.3% of the reported paediatric cases were tested by culture, demonstrating suboptimal diagnostic practices; of these, 39.9% were culture positive. The overall culture confirmation rate was 16.9% over the 9-year period of the report (but 19.2% in 2009). The mortality rate was 1.6% of all culture confirmed cases and the overall treatment outcome was successful in 75%.

Transmission within a community is measured by the annual risk of infection [5]. As 95% of children develop disease within 12 months of being infected, recording data on childhood TB give an indication of recent transmission within communities, and confirms that TB infection and disease in children are sentinel events indicating the burden of TB and the effects of control strategies in a community. Infection rates rise with increased exposure, with the highest probability of developing infection between the ages of 5 and 7 years in association with school and increased social mobility. Annual risk of infection is traditionally estimated by use of childhood tuberculin surveys, although this has limitations resulting from the poor specificity of the tuberculin skin test (TST), particularly where bacille Calmette-Guérin (BCG) vaccine is given at birth and non-tuberculous mycobacteria (NTM) are endemic. T-cell-based interferon-γ release assays (IGRAs) could offer a more specific alternative, but have not yet found a use in this context because of their high cost, ethical concerns about venepuncture in healthy children, and uncertainty about the association between a positive result and later development of active disease.

A decrease in the number of childhood cases, especially in children under 5 years of age who are mostly infected in their households, would be the first indication that transmission is decreasing in a community, emphasising the importance of the recording of TB in children. In the EU/European Economic Area, notification rates in this age group appear to be decreasing [3].

The majority of very young children (<3 years) are infected by a household source case and prudent public health policy should encourage active case finding amongst household contacts of young children with proven infection or disease. This type of ''reverse'' contact tracing can be expanded to children of all ages in low-prevalence areas, where household exposure is the most probably the source and is recommended by the WHO.

It is estimated that the majority of children (60–80%) exposed in a household to a sputum-smear positive case of TB become infected [4]. The probability varies with age, immunological status, environmental factors and socioeconomic status, as well as the duration and proximity of exposure and infectiousness of the contact. The risk of developing disease following infection is greatest in the immediate period following infection and declines with time; 95% of children develop disease within 12 months of being infected. The risk of developing disease is influenced by age, and nutritional, vaccination and immunological status. The progression from infection to disease, and particularly severe disease such as disseminated TB and TB meningitis, is substantially higher in infants and young children; most probably due to an immaturity in the cellular immune response, although factors such as host genetics, microbial virulence and impaired immune competence due to HIV or malnutrition can also play a role, particularly in resource-poor settings. Some active contact tracing studies including chest radiography have identified radiographic changes in 50–60% of children who underwent TST conversion. The majority of these were transient changes and the children did not develop disease, suggesting disease was possibly controlled by the host immune response. These data have implications for case definitions based on radiological findings alone [6].

Based on historical data, the highest risk of TB-related mortality following primary infection occurs during infancy (5–10% in natural history studies); this risk declines to 1% between the ages

of 1–4 years and <0.5% from 5 to 14 years, before increasing to adult levels of 2% from 15 years of age upwards [7].

Differences in the childhood immune response to TB

As mentioned earlier, very young children are usually infected by their caregiver and it would appear that the determining factor for the higher susceptibility to disease in children is prolonged, intimate contact between the child and the index case, which might lead to a larger inoculum of *Mycobacterium tuberculosis*. However, there is little evidence to support this assumption, since the mycobacterial load in children is notoriously low, which lies at the root of the problem of bacteriological confirmation of primary TB. It therefore appears that even low bacillary loads in very young children can lead to acute and severe illness, be it respiratory or disseminated. The generally accepted assumption is that qualitative and quantitative differences in the immune responses to *M. tuberculosis* infection between adults and children determine outcome. As described elsewhere, the host immune response to *M. tuberculosis* infection involves both the innate and adaptive immune system, starting with antimicrobial peptides and neutrophils, followed by the interaction between the antigen presenting cells and the bacteria and granuloma formation, followed by a more targeted approach by CD4 and CD8 T-cells [8]. There is a paucity of studies examining the differences in age-related immune responses to TB, but a number of recent reviews have examined the current literature [8, 9]. Neutrophils are rapidly recruited to the site of mycobacterial infection, but studies have shown conflicting reports of their contribution to protection and pathology to date [10]. Following inhalation of mycobacterial bacilli, these are taken up by antigen presenting cells (macrophages and dendritic cells), processed and presented to T-cells causing proliferation and activation of T-helper (Th) type 1 cells in particular. A comparison of infant and adult *M. tuberculosis*-infected macrophages demonstrated poorer phagocytosis, but similar intracellular killing [11]. Additionally, neonates have fewer dendritic cells and their ability to synthesise interleukin (IL)-12 is impaired [12]. These functional impairments lead to poor T-cell priming and consequently impaired immunity to TB. CD4 T-cells are known to be of importance in protection against TB and children with TB have lower interferon (IFN)-γ responses to *M. tuberculosis* than children with latent TB infection (LTBI). Such responses are further impaired in severe disease. Other T-cells, including CD8, $\gamma\delta$, Th17 and regulatory T-cells have been studied in the context of TB infection, but comprehensive data are lacking and these paediatric studies are currently ongoing.

Clinical spectrum of disease

The clinical spectrum of TB disease in children is diverse and spans from primary disease which is cleared without treatment, to severe forms such as miliary TB and TB meningitis.

Whilst the majority of immunocompetent children with primary infection do not progress to disease, young children aged <2 years and adolescents have an increased rate of progression and death, with the highest risk seen in infancy (table 1) [4]. Intrathoracic disease manifestations are the most common at all ages; however, disseminated TB or TB meningitis represent a considerable burden of disease in infants. School-aged children have the lowest rates of disease. Age is therefore a vital determinant of disease manifestation.

Immune status also influences the rate and severity of clinical disease; HIV-infected children or those on immunosuppressive therapy have increased rates of TB disease and an increased risk of severe disease [13]. In common with adults, initiation of highly active antiretroviral treatment (ART) can be associated with the development of immune reconstitution inflammatory syndrome (IRIS).

Intrathoracic pulmonary disease is the most common disease manifestation and has four potential outcomes: 1) *M. tuberculosis* may be contained and the child may be asymptomatic;

Table 1. Average age-specific risk of progression from primary infection to disease

Age at primary infection years	No disease	Pulmonary disease	Disseminated TB/ TBM	Most common disease manifestation
<1	50	30–40	10–20	Pulmonary: Ghon focus, lymph node or bronchial
1–2	75–80	10–20	2.5	Pulmonary: Ghon focus, lymph node or bronchial
2–5	95	5	0.5	Pulmonary: lymph node or bronchial
5–10	98	2	<0.5	Pulmonary: lymph node, bronchial, effusion or adult type
>10	80–90	10–20	<0.5	Pulmonary: effusion or adult type

Data are presented as %. TB: tuberculosis; TBM: tuberculosis meningitis. Reproduced and modified from [7] with permission from the publisher.

2) parenchymal disease with associated intrathoracic adenopathy; 3) progressive primary disease with caseation and cavity formation; or 4) reactivation in adolescence. Pleural effusions complicate pulmonary TB in up to 40% of children [14]. Disease progression can result from either poor or over-exuberant containment. In young infants and in immunocompromised individuals, poor containment with unrestrained proliferation of *M. tuberculosis* causes parenchymal breakdown and an increased risk of dissemination. In adolescence, an over-exuberant immune response results in adult-type cavetating disease. Children over the age of 10 years with adult-type cavetating disease are frequently smear positive and, therefore, represent a transmission risk to the community [15].

Of the extrathoracic manifestations, superficial lymphadenopathy is the most common. Left untreated, it can caseate and spread to other structures through sinus tracts. Disseminated forms such as miliary TB are not common but are severe and are frequently associated with multi-organ involvement. Central nervous system disease is uncommon but complicates miliary TB in up to 50% of cases. Lumbar punctures and imaging of the central nervous system should, therefore, be carried out in such cases. High levels of mortality or long-term neurological sequlae are associated with central nervous system disease. Manifestations, such as skeletal, abdominal, skin and renal manifestations, are less common but ought to be considered, especially in immunocompromised and/or very young children.

Diagnostic difficulties and differences in children

Despite recent advances in TB diagnosis, it remains a huge challenge in children. TB can mimic many common childhood diseases, including pneumonia, generalised bacterial and viral infections, malnutrition, and HIV. However, the main impediment to the accurate diagnosis of active TB is the paucibacillary nature of the disease in young children, and an accelerated disease progression adds to an already urgent need for rapid diagnosis. Bacteriological confirmation is the exception rather than the rule. Consequently, unlike adults, the standard for diagnosis in children has been based on clinical history, TB contact history, TST and radiological findings rather than microbiological confirmation. A number of scoring systems or algorithms have been developed to improve and standardise diagnosis, with mixed success. A recent evaluation of nine structured approaches identified a difference in case yield that ranged from 6.9% to 89.2% and concluded that although they may have some use as screening tools, they were inadequate for identifying definite TB [16]. In particular, these scoring systems are poorly adapted for the highest risk groups, HIV-infected children and children under 3 years of age, both of whom are particularly at risk of rapidly developing severe disease. The TST is an important component of many scoring systems; however, it lacks sensitivity and specificity. Furthermore, radiological findings can be difficult to interpret, and wide inter- and intra-observer variability is well described. Radiological evidence of pulmonary TB usually includes lymphadenopathy (hilar or mediastinal) and lung

parenchymal changes. The most common parenchymal changes are segmental hyperinflation, atelectasis, alveolar consolidation, pleural effusion/empyema and, rarely, a focal mass. Cavitation is rare in young children but is more common in adolescents, who may develop adult-type post-primary disease. Miliary TB is characterised by fine bilateral reticular shadowing.

Computed tomography (CT) imaging may be helpful in demonstrating pulmonary disease such as endobronchial disease, early cavitation and bronchiectasis following pulmonary TB where chest radiographs are normal or unhelpful. CT imaging, and increasingly magnetic resonance imaging (MRI), is also useful in investigating central nervous system disease, such as TB meningitis, tuberculoma or bone abnormalities. Ultrasound of the abdomen may identify lymphadenopathy, hepatic/splenic lesions or ascites.

The efficacy of diagnostic testing depends on both the quality of the sample and, most importantly in children, a high index of suspicion. Unlike adults, children often swallow rather than expectorate sputum and young children are unable to produce a sample upon request. Traditionally this has led to the collection of samples directly from the stomach in the form of a gastric aspirate or lavage. In order to maximise yield, samples are collected following an overnight fast on three consecutive mornings, which has obvious disadvantages. An alternative method of obtaining a lower respiratory sample is sputum induction. A bronchodilator is inhaled, followed by nebulisation with hypertonic (3–5%) saline and then collection of secretions by suction or expectoration in co-operative older children. There are advantages to induced sputum including no requirements for an overnight fast as it can be conducted at any time of the day following fasting for only 2–3 hours, potentially as an outpatient procedure. A number of studies have demonstrated that this collection method is effective, well tolerated and has low adverse event rates, even if there is moderate-to-severe lung disease, with limited disease spread if effective infection control measures are applied [17–19]. A comparative study showed an equivalent diagnostic yield between gastric lavage and induced sputum in a mixed population of children either with suspected TB or those exposed to a household contact. Collection of one gastric lavage and one induced sputum specimen on the same day had a similar yield to two consecutive day gastric lavage collections. This may represent a practical diagnostic approach with appropriate infection control measures in place. It is vital to increase the acceptability and rate of sample collection in children with suspected TB and to overcome the perception amongst health workers that a microbiologically confirmed diagnosis is neither possible nor useful in children, especially in the day and age of potentially drug-resistant (DR)-TB.

Other methods of sputum collection have also been investigated in children, such as bronchoalveolar lavage (BAL), the string test and nasopharyngeal aspirate, with limited improvement over gastric aspirate/induced sputum [20–22]. Bronchoscopy, with an experienced operator, enables visualisation of the bronchial tree, which can provide clues such as caseation, allows transbronchial lymph node biopsy and may also provide an alternative diagnosis. It should not be recommended for routine diagnosis of TB, but has a role in intubated children or if otherwise clinically indicated.

As most children swallow their sputum, mycobacterial DNA may survive the transit of the gastrointestinal tract allowing molecular testing of stools. Initial work in Peru demonstrated a low sensitivity and high specificity of stool PCR; however, a further study using GeneXpert® MTB/RIF (Cepheid, Sunnyvale, CA, USA) was more promising with 100% sensitivity in stool samples, but the sample size was small and further studies are required [23]. TB lymphadenitis is a common form of extrathoracic TB and children with pulmonary TB have concomitant TB lymphadenitis in 10–30% of cases; fine-needle aspirate biopsy of accessible cervical or axillary lymph nodes has been shown to have both a greater yield and a decreased time to bacteriological diagnosis compared to other specimens (7 days compared to 22 days) [24]. Further paediatric studies, including the use of GeneXpert® on these samples is warranted.

Commercial PCR tests are showing increasing promise for the diagnosis of TB and the GeneXpert® MTB/RIF, an integrated sample processing and nucleic acid amplification test for

detection of *M. tuberculosis* and resistance to rifampicin, was recently studied in a large South African paediatric study comparing the yield on induced sputum to a standard reference of liquid mycobacterial culture [25]. One sample produced a sensitivity of 84.6% for smear-positive disease and 33.3% for smear-negative disease. The yield was doubled with the addition of a second sputum sample, increasing the sensitivity by 28% to 61.1%. A second study in Tanzania of 164 children of whom 51% were HIV infected showed a sensitivity of 66.6% in smear-negative/culture-positive children. They did not identify any difference in performance due to HIV infection [26]. The results of both studies suggest the benefits of multiple-sample testing, which is not currently recommended by WHO guidelines, but this would need to be balanced against the increased costs. Culture remains important and, in fact, is more sensitive than the GeneXpert® for both identifying those children who are PCR negative but culture positive and for complete drug-susceptibility testing (DST) in those children with DR-TB. A recent Italian study of the GeneXpert® in extrapulmonary (EP) TB, using a variety of samples including pleural fluid, cerebrospinal fluid, gastric aspirates, biopsies, urine, pus and FNA samples including 494 out of 1,476 samples from paediatric cases, showed great promise. GeneXpert® had a higher sensitivity and specificity in paediatric compared to adult samples. The highest sensitivity for paediatric samples was in biopsies, pus or pleural fluid while gastric lavage and cerebrospinal fluid had a sensitivity of 81% and 75%, respectively, compared to culture and clinical diagnosis [27]. A meta-analysis of the performance of GeneXpert® for diagnosis of EPTB reported an overall pooled sensitivity of 80% and a specificity of 86% compared to a gold standard of culture [28].

The TST and IGRAs are immunology-based diagnostic tests of mycobacterial sensitisation. It is important to remember that neither test proves the presence of mycobacteria and that IGRA were not designed for the diagnosis of active TB but rather as an indicator of LTBI. They have, however, been widely applied to diagnose active TB. Two meta-analyses to appraise their role in the diagnosis of TB disease in children have recently been published [29, 30]. MACHINGAIDZE *et al.* [30] included 20 studies and looked at the performance of the QuantiFERON®-TB Gold In-Tube (QFT-GIT; Cellestis, Carnegie, Australia) assay exclusively. MANDALAKAS *et al.* [29] included 32 studies with a total of 4,122 children and assessed both the QFT-GIT and T-SPOT®.*TB* test (Oxford Immunotec, Oxford, UK). Both identified that the sensitivity of the IGRA for TB disease was similar to the TST, with lower sensitivities reported for high-burden TB countries. The specificity for detection of TB disease was 91% for QFT-GIT and 94% for T-SPOT®.*TB* (compared to 88% for TST). All diagnostic assays, the TST, QFT-GIT and T-SPOT®.*TB*, had reduced sensitivity in three groups: those aged <5 years, HIV-infected individuals and those with BCG vaccination rates $>50\%$. Individual studies reviewed in the latter meta-analysis found associations between indeterminate results and young age, helminth infections and immune suppression, although significant associations could not be confirmed in the stratified analysis. At best, the IGRAs represent a rule-in rather than a rule-out test for TB disease; using TST and IGRAs in combination can increase sensitivity to up to 93% [31] and should be interpreted as an additional piece of evidence. However, given the current evidence, WHO has not endorsed their use for the diagnosis of active TB.

However, differences appear to exist depending on the epidemiological context. MANDALAKAS *et al.* [29] found increased sensitivity for diagnosing LTBI in countries with a TB incidence $\leqslant 25$ per 100,000. Sensitivity in low- *versus* high-incidence settings was 83% *versus* 68%, 86% *versus* 68% and 87% *versus* 73% for TST, QFT-GIT and T-SPOT®.*TB*, respectively [29]. In the 2010 updated guidelines, the Centers for Disease Control and Prevention caution against the use of IGRAs in children less than 5 years of age. Lack of performance data, potential for progression to disease and concern of attenuated IFN-γ responses in this age group influenced this decision. The 2011 guidelines from the National Institute for Health and Clinical Excellence (NICE) in the UK recommend a dual strategy (IGRAs should be used to confirm a positive TST) for diagnosing LTBI in children aged 5–15 years. In children younger than 5 years of age, the TST is recommended. However, in household contacts of TB cases aged 2–5 years of age, when the initial TST is negative, an IGRA may be used along with a TST in repeat testing to increase sensitivity. In outbreak situations, IGRAs can be used alone for those older than 5 years of age. GRAHAM [32]

identified a low level of indeterminate results from IGRAs overall (1.8%) in a study of 1,128 European children assessed for LTBI, but it was 3.6% in the children younger than 5 years of age. The concordance between the IGRA and TST was fair (k=0.34) and both age and BCG vaccination record correlated with positivity of TST. In this cohort, BCG vaccinated children were less likely to have a positive IGRA, indicating a possibly protective effect of BCG vaccination against TB infection. Of note, when the TST was interpreted as negative, a positive IGRA was found in between 3.5% and 23.9% of cases depending on the TST cut-off, highlighting the importance of performing an IGRA in both TST-positive and -negative individuals, if used as part of guidelines.

Treatment of TB in children

In general, the principals of treatment and recommended regimens are entirely derived from adult regimens, since, unfortunately, there are no studies of TB drug efficacy in children with TB. Therefore, the same four-drug regimen (HRZE (isoniazid-rifampicin-pyrazinamide-ethambutol)) for 2 months followed by a two-drug regimen (HR) for 4 months is recommended. A three-drug regimen (without ethambutol) for 2 months followed by HR for 4 months can also be used for HIV-negative children with suspected or confirmed pulmonary TB or tuberculous peripheral lymphadenitis who live in settings with low HIV prevalence or low isoniazid resistance. The exception is TB of the central nervous system, bones and joints for which 12 months of treatment with a four-drug regimen (HRZE) is recommended in the first 2 months, followed by HR for 10 months. Several recent studies and re-analysis of previously published studies have led to changes in dosing guidelines for several of the first-line anti-TB drugs, notably isoniazid and ethambutol. In general, these studies have shown that for children older than 3 months of age, larger per kilogram doses are required to achieve adequate serum levels of the drug (table 2). Remarkably, there remains a dearth of data for determining optimal drug doses in infants. The absence of child-friendly fixed dose combinations of TB medications is a challenge in many settings.

Multidrug-resistant/extensively drug-resistant TB

Multidrug-resistant (MDR)-TB is defined as *M. tuberculosis* resistant to the most potent first-line anti-TB medications, isoniazid and rifampicin, while extensively drug-resistant (XDR)-TB has additional resistance to the most active second-line agents, injectable drugs (aminoglycosides and/or cyclic polypeptides) and fluoroquinolones. Since most children are treated for primary TB, DR-TB is usually acquired from infection with an already drug-resistant strain, rather than developing due to poor adherence to therapy. In children, there is an increased risk of DR-TB infection in the following circumstances: 1) exposure to a known drug-resistant case; 2) exposure to a case who has had treatment failure or relapse; 3) exposure to a case who remains sputum smear-positive

Table 2. Changes in the dosing of first-line anti-tuberculosis drugs for children

Drug	Previous daily dosing (range)[#] mg·kg^{-1}	Revised daily dosing (range)[¶] mg·kg^{-1}
Isoniazid[+]	5 (4–6)	10 (10–15)
Rifampicin		
0–3 months	10 (8–12)	No change
>3 months	10 (8–12)	15 (10–20)
Pyrazinamide		
0–3 months	25 (20–30)	No change
>3 months	25 (20–30)	35 (30–40)
Ethambutol	20 (15–25)	No change

[#]: based on the 2006 World Health Organization guidelines; [¶]: based on the 2010 World Health Organization guidelines; [+]: recommended for prophylaxis and treatment. Reproduced and modified from [32] with permission from the publisher.

Table 3. Adverse effects associated with first- and second-line anti-tuberculosis agents

Drug	Main adverse effects
Isoniazid	Hepatitis, peripheral neuropathy
Rifampicin	Hepatitis
Pyrazinamide	Hepatitis
Ethambutol	Optic neuritis
Kanamycin/amikacin/capreomycin	Ototoxicity, nephrotoxicity
Fluroquinolones	Sleep disturbance, GI disturbance, arthritis, peripheral neuropathy
Ethionamide/prothionamide	GI disturbance, hypothyroidism, metallic taste
Cycloserine/terizidone	Neurological and psychological effects
PAS	GI disturbance, hypothyroidism, hepatitis
Clofazimine	Skin discolouration
Linezolid	GI disturbance, headache, myelosuppression, neurotoxicity, lactic acidosis, pancreatitis
Clarithromycin	GI disturbance, rash, hepatitis, prolonged QT syndrome, ventricular arrhythmias
Thiacetazone	Stevens–Johnson syndrome in HIV-infected patients, GI disturbance, hepatitis, skin reactions

PAS: para-aminosalicylic acid; GI: gastrointestinal. Data from [34].

after 2 months of therapy; 4) exposure to a case from an area with high prevalence of resistance; and 5) travel to an area with high drug resistance [33]. Both DST and rates of resistance have increased in the EU/European Economic Area over the last 10 years [3].

Few studies have examined the management of children with MDR-TB. Children are typically diagnosed with either confirmed or, more often, presumed MDR-TB in the absence of available culture confirmation. Treatment in such cases should be based on the DST of the confirmed index case, or the source of the presumed case. If no DST is available and the child is failing therapy, treatment decisions should be based on the prevailing DST pattern of MDR-TB strains circulating in the region. Designing a treatment regimen is based on the same recommendations as for adults, using at least four drugs, preferably five, to which the organism is susceptible.

Additional challenges to treating MDR-TB in children arise from the uncertainty about activity and safety of the available drugs. The second-line drugs are rarely produced in paediatric formulations or appropriate tablet sizes, necessitating breaking, splitting, crushing or grinding. Hence dosing may be inaccurate and sub-therapeutic or toxic levels are possible. The taste of the medications is often unpalatable. A number of the drugs cause vomiting and diarrhoea that may affect the amount absorbed and possibly sub-optimal doses. The daily pill burden can be vast as the child may require multiple TB medications plus ART and other antibiotics, as well as supplements of vitamins and calories in some settings.

The pharmacokinetic parameters of most of the second-line anti-TB agents in children of various ages are unknown, and optimal regimens for specific patterns of drug resistance are undefined. This is an important area for further research, since licensing of formulations in Europe now require a paediatric investigation plan and specific paediatric studies.

The adverse effects of the first-line medications have been well described and are less common in children than in adults. A list of the adverse effects of the anti-TB agents is given in table 3. Children should be screened for optic neuritis, oto-toxicity and hypothyroidism as appropriate whilst on treatment for MDR-TB.

TB/HIV co-infection

Expert advice should be sought for management of all children co-infected with TB and HIV. The incidence of TB disease in HIV-infected children is reported to be as high as 20 times that in

non-HIV-infected children [35]. Similar to adults, treatment outcomes for TB are poorer in children with HIV compared with HIV-uninfected children [36]. HIV-infected children also experience greater morbidity and mortality from TB disease and have an increased risk of relapse [37]. Some postulated reasons for this include underlying immunosuppression, the presence of co-infections, underlying chronic lung disease, malnutrition and poor drug absorption. Unlike other opportunistic infections, children with HIV are at risk of TB disease even when they have a relatively high CD4 count. All children diagnosed with TB should be screened for HIV. Children newly diagnosed with HIV should be screened for TB by clinical history and chest radiograph. There are conflicting reports of the role of routine isoniazid prophylaxis in children with HIV, with one study showing a decrease from 23.4% to 7.2% of TB disease compared to placebo in the absence of ART [38]. These findings were not confirmed in a larger study in the same population [39]. There were low rates of adverse events in this study and, of note, both highly active ART and isoniazid independently decreased the risk of TB disease. Contact tracing in households affected by TB-HIV co-infection is of particular importance.

Screening for TB is especially important in HIV-infected children, both because of the risk for rapid disease progression and the complexities of adding a TB therapy to a regimen of antiretroviral therapy.

The drug interactions between ART and first-line TB drugs have been extensively reviewed in adults. Rifampicin reduces the concentrations of many concomitantly administered drugs including the key antiretroviral non-nucleoside reverse transcriptase inhibitors and protease inhibitors. Low serum protease inhibitor concentrations can be partially overcome with the use of high doses of ritonavir or by doubling the usual dose of the co-formulated form of lopinavir/ritonavir, but subsequent hepatotoxicity is a common adverse effect. Efavirenz serum concentrations are lowered modestly by rifampin, but this does not appear to affect the efficacy of antiretrovirals. The combination of efavirenz-based ART and rifampin-based TB treatment, at their standard doses, is usually the preferred treatment for HIV-related TB in children older than 3 years of age. For children aged less than 3 years of age with prior exposure to a non-nucleoside reverse transcriptase inhibitor, a regimen of two nucleoside reverse transcriptase inhibitors plus super boosted lopinavir and ritonavir is recommended. Rifabutin, when available, may be substituted for rifampin when ART using protease inhibitors must be used, but the data for outcomes in children are extremely limited. A complete review of recommended approaches to managing dual HIV/TB infections in children can be found in the 2010 WHO guidelines.

IRIS is characterised by an acute worsening of signs and symptoms of disease that occurs with immune recovery. In the setting of HIV, this correlates with the start of antiretrovirals. IRIS has been associated with the following risk factors (significant immunosuppression as measured by CD4 count, HIV viral load, severe TB disease, and early start and rapid response to antiretrovirals) and is found in up to 29% of cases in children with low CD4 counts. Due to the potential for IRIS in these risk groups, it is recommended that ART be delayed by 2–8 weeks after the start of anti-TB therapy in all children with EPTB. Additionally, antiretrovirals should be delayed by 2–8 weeks in children with pulmonary disease or lymphadenitis with a CD4 count below the WHO threshold. In children with pulmonary disease or lymphadenitis with CD4 counts above the threshold that have a good response to TB therapy, antiretrovirals can be started once TB therapy is completed. It should be remembered that IRIS can occur due to BCG or NTM and is not necessarily TB.

TB control and prevention

The BCG vaccine is the only currently licensed TB vaccine. It is a live attenuated vaccine that is administered at or near birth in most countries worldwide [40] and has been in existence since 1921. It became integrated into the Expanded Programme on Immunization infant vaccination schedule in 1974.

The BCG is administered to an estimated 100 million infants worldwide every year, making it one of the most widely used of all vaccines [41]. Whilst it affords significant protection against disseminated disease and TB meningitis, the protection against pulmonary TB is inconsistent, with the most limited protection in geographical areas where TB is most prevalent [41, 42]. The poor protection against adult-type disease is evident since the global TB epidemic has occurred despite the majority of the world receiving the BCG.

BCG vaccination of HIV-infected children is associated with disseminated BCG disease and the benefit conferred is questionable; the BCG is therefore no longer recommended in individuals known to be infected with HIV [43].

The need for a new vaccine is evident and urgent. There are 14 candidate vaccines currently in clinical trials; however, the challenges facing the development of new vaccines are significant [44]. The aim is to produce a vaccine that is efficacious against all forms of TB in all age groups and that is safe in the context of HIV infection. A further obstacle to the vaccine pipeline is that there is no defined correlate of protection against TB; clinical trials currently measure vaccine ''take''. Using clinical disease as an end-point necessitates large sample sizes and prolonged follow-up. Active case finding, prophylaxis of household contacts and effective treatment coupled with a paucity of highly sensitive and specific diagnostic tools makes this task very difficult.

The main vaccine approaches are: 1) pre-exposure vaccines, which aim to prevent infection and primary disease prior to exposure; 2) post-exposure vaccines, which aim to prevent reactivation after infection; and 3) immunotherapeutic vaccines, which could be used to shorten duration of TB treatment.

Pre-exposure vaccines are most likely to be of benefit to infants and young children, where the highest rate of progression from primary infection to disseminated disease is seen. Such vaccines may not eradicate *M. tuberculosis* but rather elicit a cell-mediated immune response containing *M. tuberculosis* at the site of primary infection. They employ a heterologous ''prime-boost'' strategy [45]. BCG is retained due to efficacy against severe forms of childhood TB or a recombinant BCG vaccine may serve as the priming vaccine. A second boost vaccine is then used to expand memory T-cells common to the prime and boost vaccines. There are two such vaccines currently in phase IIb clinical trials enrolling large numbers of infants: MVA85A/Aeras485 and AERAS-402/Crucell Ad35 [46, 47]. Another adjuvant recombinant fusion protein vaccine, M72, is currently in phase II trials. Post-exposure and immunotherapeutic vaccines are more likely to be used in adolescence to prevent reactivation of latent disease.

Whilst a new improved TB vaccine in early childhood is important to prevent the burden of disease in children, it is unlikely to effect a rapid reduction in TB incidence. Rather, the combination of a pre- and post-exposure vaccine administered in mass campaigns, targeting adults, it most likely to achieve a substantial effect [44]. Prevention of TB or a shortened period of infectivity in adults would have the benefit to children of preventing transmission and thus would protect infants and children as well. The ideal vaccine for TB control should also be able to prevent infection.

Although primary prevention, giving isoniazid to young children recently exposed to a case of TB, has been recommended by the WHO for decades, it has rarely been used in the high-burden settings of disease. There are conflicting data on the role of primary prevention in children with HIV infection who live in high-burden TB settings. It is becoming clear that the IGRAs may have great utility in low-burden settings,

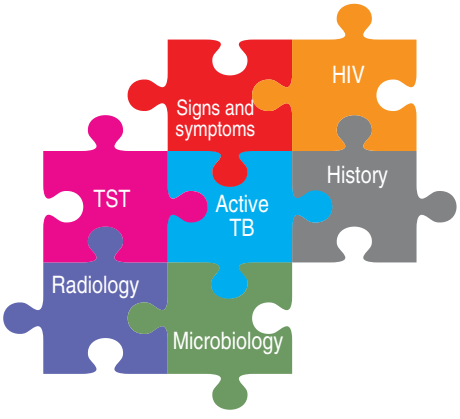

Figure 1. The ''pieces of the jigsaw'' that might be required to make a diagnosis of childhood tuberculosis (TB). TST: tuberculin skin test.

especially for children who have received the BCG vaccine, where specificity in detecting TB infection (to avoid false-positive TST results and unnecessary treatment of patients) is important [48]. Finally, although the standard 6 months of isoniazid therapy for TB infection in children is very safe and effective, adherence rates are often very low. The role of chemoprophylaxis in the setting of MDR-TB is less clear and opinions differ as to whether to watch closely and wait once disease has been ruled out by clinical examination and chest radiograph, or to give at least two drugs to which the strain of a presumed index case is sensitive. However, there are no evidence-based guidelines at present and paediatric household contacts should be closely monitored in an outpatient setting for 2 years. Further research is urgently required to determine the right approach at a given age group and potential length of prophylactic therapy.

In summary, childhood TB remains a challenge for clinicians, epidemiologists and researchers with progress required in all three main areas: prevention, diagnosis and therapy.

However, it is important to consider the diagnosis of TB in children in the context of any family or known TB contact, and to carefully evaluate all children potentially affected. In the absence of bacteriological confirmation, a ''jigsaw approach'' might be required, as illustrated in figure 1.

International Standards for Tuberculosis Care and EU Standards for Tuberculosis Care have made the current research relevant and accessible to the practicing clinician to ensure optimal diagnosis, treatment and prevention of TB [49, 50]. However, given the specific challenges of childhood TB, a set of specific European standards of care for children is still outstanding. To make further progress in practical management and research and advocate for childhood TB we must continue to raise the profile of children and families affected by TB in European and international forums and *via* networked activities [51].

Statement of Interest

B. Kampmann's laboratory has previously received a discount on consumables to conduct either IGRA from both Cellestis (Carnegie, Australia) and Oxford Immunotec (Oxford, UK). However, neither company has ever been involved in the analysis or interpretation of the data.

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Chapter 16

TB as an occupational disease

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SUMMARY: Tuberculosis (TB) in healthcare workers (HCWs) and TB in combination with silicosis are considered occupational diseases. Improved hygiene in the workplace has led to a reduction of exposure to mineral dust. Therefore, the incidence of silicosis, as well as silicotuberculosis, in high-income countries has declined. However, silicotuberculosis remains a significant and frequently occurring occupational disease in low- and middle-income countries. Recent studies have reported mortality ratios of between 2.2 and 27. Even in high-income countries the risk of TB in HCWs is increased for a wide range of tasks in healthcare, and the prevention of nosocomial infection of HCWs remains a challenge. Interferon-γ release assays (IGRAs) facilitate the screening of HCWs. In comparison with the tuberculin skin test (TST), the IGRAs reduce the number of radiographs and the amount of chemoprevention needed. However, a grey zone should be introduced for the interpretation of IGRA results in the serial testing of HCWs.

KEYWORDS: Healthcare worker, interferon-γ release assay, risk assessment, screening, serial testing, tuberculosis

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Healthcare workers (HCWs) are exposed to infectious agents. The excess death rate of HCWs due to work-related infections was estimated to be between nine and 24 deaths per million healthcare workers in the USA [1]. However, the increased risk of infection for HCWs is not always easy to detect. This not only pertains to new, emerging infectious diseases like severe acute respiratory syndrome (SARS) [2], but also to well-known diseases like tuberculosis (TB). For a long time, working in healthcare was considered a safe environment offering protection against TB. Only when the prevalence of the disease declined in the general population did it become apparent that the rate of latent TB infection (LTBI) and active TB was high in those caring for TB patients [3]. With the further decline of TB in high-income countries, interest in TB as an occupational disease dwindled. It was with the emergence of HIV that interest in TB as a co-infection inspired research and fostered the prevention of infectious diseases in HCWs. As the increased risk of TB in HCWs is well known, TB in HCWs is on the list of occupational diseases compiled by the International Labour Organization [4]. TB in HCWs can be accepted and compensated as an occupational disease in all countries of the European Union (EU) [5]. The legal

requirements for TB to be accepted as an occupational disease in a HCW vary. In some countries, working in healthcare is considered as proof of exposure (*e.g.* France and Portugal) while in other countries working in healthcare is not enough to fulfil the exposure requirements (*e.g.* Germany). Here, in addition to working in healthcare, it needs to be proved that the HCWs with active TB had contact with TB patients or infectious materials, or performed tasks with high risk of exposure to *Mycobacterium tuberculosis* (*e.g.* bronchoscopy, resuscitation or intubation) in order to meet the legal requirements for TB in a HCW to be accepted as an occupational disease.

However, TB as a work-related disease can occur in another guise. The increased risk of TB in miners is well known, and silicotuberculosis is therefore considered an occupational disease in most EU countries, *e.g.* Austria, Belgium, Germany, Finland, France, Italy, Luxembourg, the Netherlands, Portugal, Spain and the UK [5].

TB screening in HCWs is performed in order to prevent nosocomial transmission from HCWs to patients, and in order to detect and treat recent LTBI in HCWs [6]. Screenings can be performed as a pre-employment screening, a routinely repeated screening or as contact tracing after accidental contact with infectious patients or materials. Therefore HCWs undergo repeated TB screening, and the interpretation of results in the serial testing of HCWs is a significant issue. For many years these screenings have been performed using the tuberculin skin test (TST). For several years now, two interferon-γ release assays (IGRAs) have been commercially available: the ELISA-based QuantiFERON®-TB Gold In-Tube (QFT-GIT; Cellestis, Victoria, Australia) and the ELISPOT-based T-SPOT®.*TB* (Oxford Immunotec, Abingdon, UK). They are currently being evaluated for use in the serial TB screening of HCWs [7, 8].

In this chapter we present an overview of the different aspects of TB as an occupational disease. Emphasis is placed on the situation in Europe; however, as silicotuberculosis is of decreasing importance here, the focus in this regard will be on Africa. Furthermore, the performance of the IGRA in comparison with the TST in TB screenings of HCWs will be presented. Special emphasis will be placed on the IGRA results in the serial testing of HCWs.

Literature search

Recent reviews and studies which will update these reviews were identified from the PubMed and EMBASE databases. In addition, published data from French [9], Portuguese [10, 11] and German HCWs [12–14] will be summarised here.

Search results

Several well-performed reviews covering different aspects of TB in HCWs are available. Baussano *et al.* [15] assessed the annual risk of LTBI and the incidence rate ratio (IRR) for TB among HCWs using the incidence rate of the country in which the original study was performed as a reference. Three strata were defined: 1) countries with a low incidence of TB (<50 cases per 100,000 population); 2) countries with an intermediate incidence of TB (50–99 cases per 100,000 population); and 3) countries with a high incidence of TB (>100 cases per 100,000 population). The incidence of LTBI has been defined as tuberculin conversion after a documented negative baseline (TST). The pooled estimates for the annual risk of LTBI were 3.8%, 6.9% and 8.4% for low-, intermediate- and high-incidence countries, respectively. The pooled annual TB IRRs were similar for low- and intermediate-incidence countries (2.42 and 2.45, respectively). In countries with a high incidence of TB, the IRR was higher (3.68). While for the incidence of LTBI an association with the incidence of TB in the general population is observed, the same is not true for the IRR for TB in HCWs. The IRR for low-incidence countries (defined as <50 cases per 100,000 population) are based on four studies, one being a study from Portugal [10]. In this study, the highest IRR was observed (5.99). In a later publication, the same working group reported a steady decline of TB in HCWs once systematic screening was started [11]. Therefore, the pooled IRR for

the low-incidence countries overestimates the risk of TB in HCWs. In summary, BAUSSANO *et al.* [15] demonstrate an elevated risk of LTBI and TB regardless of the country's incidence of TB in the general population.

SEIDLER *et al.* [16] analysed task-specific infection risks in healthcare in low-incidence countries. Summarising their findings the authors conclude that the available epidemiological evidence is given for an elevated risk of TB in the following occupational groups in low-incidence countries: hospital employees in wards with TB patients; nurses in hospitals; nurses caring for HIV-positive or drug-addicted patients; pathology and laboratory workers; respiratory therapists and physiotherapists; physicians in internal medicine, anaesthesia, surgery and psychiatry; non-medical hospital personnel in housekeeping and transport; funeral home employees and prison employees.

JOSHI *et al.* [17] performed a systematic review of LTBI and TB among HCWs in low- and middle-income countries. In summary, the prevalence (between 33% and 79%) and incidence (between 0.5% and 14.3% per year) of LTBI and the risk attributable to TB due to nosocomial exposure (from 25 to 5,361 cases per 100,000 per year) were high among HCWs in low- and middle-income countries. The attributable risk was higher in healthcare facilities that had more TB patients per HCW. Certain work locations (inpatient TB facility, laboratory, general medicine and emergency facilities) and occupational categories (radiology technicians, patient attendants, nurses, ward attendants, paramedics and clinical officers) were associated with a higher risk of TB.

MENZIES *et al.* [18] analysed the incidence of LTBI and active TB in HCWs in low- and middle-income countries and in high-income countries. In accordance with the findings of BAUSSANO *et al.* [15] the authors report an increased risk of LTBI and active TB in low- and middle-income countries, as well as in high-income countries. However, the authors point out that only a few studies of the incidence of TB in HCWs in high-income countries are available and that it becomes increasingly difficult to carry out these studies in high-income countries as the incidence of TB in HCWs in these countries has become lower and not all HCWs can be assumed to be at increased risk any more. Furthermore, it can no longer be expected that the incidence rate of TB in HCWs in high-income countries with high infection control standards is higher than the rate in the general population. The healthy worker effect and the protective effect of a high socioeconomic status should be considered. Therefore, analysing the relative risk of TB in HCWs gets more complicated as an appropriate control group, which is comparable to HCWs in respect to the socioeconomic status, needs to be defined.

Cluster analysis is a potential measure to circumvent this problem. Cluster analysis is based on the possibility to distinguish different strains of *M. tuberculosis via* DNA genotyping. Only persons who belong to a cluster of identical fingerprints are potentially in an infectious chain. However, whether transmission within the cluster is really likely needs to be confirmed by questioning the members of a cluster about potential contacts to the other members in the cluster. These analyses allow the estimation of the proportion of TB in HCWs which is caused by infections at the workplace. So far there are two studies available that followed this approach. DEVRIES *et al.* [19] analysed TB in Dutch HCWs. Following their data, approximately every second case of TB in a HCW (42%) is caused by exposure in the workplace. DIEL *et al.* [20] identified 10 HCWs in their database of 848 fingerprints in Hamburg, Germany. In eight out of these 10 HCWs a nosocomial infection was the cause of the TB. HCWs were under represented in the cohort of TB cases from Hamburg for which a fingerprint was performed. This confirms the observation of MENZIES *et al.* [18] that the incidence rate of TB in HCWs in high-income countries is now no longer higher than the rate in the general population. However, if a HCW from a high-income country develops TB, the disease is likely to be caused by nosocomial infection.

Effects of infection-control measures

Different measures for the control of infection in healthcare have been described previously [6]. The most important are early identification and isolation of infectious patients, ventilation

control, masks for patients and respirators for HCWs in close contact with infectious patients or materials [6]. In the USA, it was well demonstrated that implementation of these infection-control measures reduces nosocomial infection. Between 1985 and 1993, several outbreaks of multidrug-resistant (MDR)-TB were reported in nosocomial settings in the USA [17]. This led to recommendations for a comprehensive set of infection-control practices to protect HCWs and reduce nosocomial transmission [6, 21]. In the years following the publication of these recommendations there was a dramatic decline in the burden of TB among HCWs [6, 21, 22].

In Italy, BAUSSANO *et al.* [23] showed that introducing infection-control measures led to a decrease in the annual rate of TB infection in HCWs. In Portugal, TORRES COSTA and co-workers [10, 11] demonstrated that the introduction of systematic screening of HCWs for TB accompanied with improved infection control reduced the incidence of TB dramatically.

TB screening for HCWs

TB screening for HCWs is considered a cornerstone for preventing TB in hospitals [6]. Screening is performed in order to exclude infectious TB in HCWs and to offer preventive treatment to HCWs with LTBI, who are likely to progress to active TB. Therefore, chest radiography is needed in HCWs with a positive immunologic test (TST or IGRA). After excluding active TB in HCWs with a positive test, preventive treatment should be considered when a recent LTBI is likely. In the most comprehensive and recent systematic review on IGRAs in HCWs [8], no data on disease progression in HCWs after a positive IGRA was reported. This might be taken as an indication that progression risk in HCWs with a positive IGRA is small. Therefore, a reluctant approach toward preventive treatment of HCWs, seems justified. However, for evidence-based recommendations, studies on disease progression in HCWs depending on variation in IGRA (conversion or reversion) are needed.

Selection of HCWs for screening should be performed based on risk assessment. Pre-employment screening is important if the recruited workforce migrated from countries with high TB incidence. The risk of exposure for HCWs can be categorised as follows: HCWs with regular contact with infectious TB patients or infectious material belong in the high-risk group, *i.e.* HCWs in TB wards and laboratories that analyse sputum or other fluids for *M. tuberculosis*. Depending on the TB incidence in patients, they might also be HCWs in emergency rooms or HIV outpatient clinics. The medium-risk group comprises HCWs with regular contact with patients who are not known to have active TB. Once again, the risk of infection here depends on the incidence of TB in the patients. All HCWs who do not have regular contact with patients or potentially infectious materials belong in the low-risk group. HCWs in the high-risk group should be screened routinely on an annual, bi-annual or tri-annual basis, depending on risk assessment. In countries with low TB incidence and high hygiene standards, screenings every 2 or 3 years might suffice instead of annual screening if the conversion rate in HCW is low. For the medium-risk group, it can be considered whether TB screening is performed routinely or is done exclusively as contact tracings. Again, this depends on the above-mentioned risk assessment. HCWs in the low-risk group should not be routinely screened because this would reduce the positive predictive value (PPV) of the screening test. They should only be screened after accidental close contact with infectious patients or materials. If screening is performed because of accidental contact, the TST or IGRA should be performed no earlier than 8 weeks after the last known contact. The first radiograph for excluding active TB after a positive IGRA should be performed 3 months after the last known contact and be repeated 9 months later [6]. Otherwise, a radiograph to exclude active TB is performed immediately after a positive IGRA in HCWs routinely screened because of risk assessment.

Good infection control can reduce infection risk even in the high-risk group [18]. Therefore, it might be discussed whether TB screening is still needed when sophisticated infection control is in place. However, as it is important to monitor infection control continuously [6], TB screening remains important even in settings with high infection control standards.

As was shown in the review of SEIDLER *et al.* [16] not only HCWs but also workers exposed to a high-incidence population for TB, such as people who work in prisons or who care for the homeless or the undocumented migrants from high-incidence countries, are at increased risk for TB infection. Again screening of these workers should be performed following risk assessment.

Until recently, TB screening was performed using a TST, which has several disadvantages, the most important being cross-reactivity with a bacille Calmette–Guérin (BCG) vaccination, booster phenomena due to intradermal application and a rather low level of sensitivity. IGRAs are a promising tool to overcome these problems [8, 24, 25]. Because the IGRAs use antigens specific to *M. tuberculosis*, they do not show cross-reactivity with BCG vaccination and most non-tuberculous mycobacteria (NTM). As IGRAs are *in vitro tests*, the problem of boosting in serial testing is circumvented. IGRAs correlate better than TSTs with exposure to infectious patients and IGRAs show a higher sensitivity for active TB than the TST [26]. Furthermore, in low-incidence countries, IGRAs have a higher predictive value for disease progression [27–29]. Therefore, IGRAs are likely to improve both the effectiveness and the efficiency of HCW screening [30]. However, interpretation of IGRA results in the serial testing of HCWs remains to be clarified and a consensus on the interpretation needs to be found [7, 8, 31, 32].

The introduction of IGRAs in TB screening of HCWs in high-income countries will reduce the number of radiographs and the amount of preventive treatments that would be needed if a decision was made based on the TST [8]. This is particularly true for countries in which BCG vaccination is still performed or was performed until recently. In a combined cohort consisting of HCWs from Portugal, France and Germany, 40.2% of the HCWs had a positive TST which was not confirmed by an IGRA [10–14]. For these HCWs, further evaluation using a radiograph can be spared, as so far there is no evidence that the prevalence of active TB or progression to active TB is increased [27] in HCWs with a positive TST and a negative IGRA. The proportion of HCWs with a negative TST but a positive IGRA is small (2.5% of all HCWs in this combined cohort). However, 10% of the HCWs with a positive IGRA did have a negative TST. Using a two-step approach, the IGRA as a confirmatory test for HCWs with a positive TST, would underestimate the prevalence of LTBI by 10% in this combined cohort. In Germany, this underestimation would be 40%, while in Portugal the underestimation would be much lower (6.5%). Therefore, two-step screening cannot be recommended for a country with a low incidence of TB, while for a country with a higher incidence of TB, the problem of underestimation seems to be on a scale that might be acceptable.

The probability of indeterminate IGRA is low in HCWs [7–14], but if it occurs then the IGRA should be repeated. In the unlikely event that the second IGRA is also indeterminate, clinical reasons for the indeterminate test should be evaluated and active TB excluded by radiography.

Neither the TST nor the IGRA are able to distinguish between a remote and a recent infection. Taking the high prevalence of positive IGRA into consideration, it is to be assumed that most positive IGRA are due to a remote infection. As progression to active TB is highest during the first 2 yrs after infection it does not seem to be useful to offer preventive treatment to all HCWs with a positive IGRA. The standard preventive chemotherapy is isoniazid for 6 or 9 months. If the suspected index patient has isoniazid-resistant TB, rifampicin for 3–4 months is recommended as chemoprevention of the HCWs with LTBI [6]. Alternatively combined short-term therapy with isoniazid and rifampicin for 3 months is possible. In European countries different regimes are performed for preventive chemotherapy in HCWs. As no data is available concerning the effectiveness of preventive chemotherapy in HCWs following a positive IGRA, the efficacy of these regimes remains to be studied.

So far, the variability of the IGRA in serial testing is not well understood. Two reviews have covered the topic of IGRA variability in the serial testing of HCWs [7, 8]. Both came to the conclusion that reversion of positive IGRA results to negative results occurs more often than conversion from negative IGRA results to positive ones. And, more importantly, the probability of conversion or reversion depends on the quantitative results of the first IGRA. Therefore, a

borderline zone might be helpful in order to separate real conversions and reversions from variation caused by chance. For the T-SPOT®.*TB*, a borderline zone of five to seven spot-forming cell is proposed by the Centers for Disease Control and Prevention and European Centers for Disease Control and Prevention. However, so far no consensus has been reached regarding the definition of such a borderline zone for the QFT-GIT. A borderline zone of 0.2 and 0.7 $IU \cdot mL^{-1}$ and defining conversion or reversion as the trespassing of this borderline zone minimised the conversion and reversion rate without inflating the proportion of HCWs falling into the borderline zone with their QFT-GIT results [7, 11, 14].

As the reversion rate in IGRAs is higher than expected [7, 8], the "once positive always positive" approach followed with TST can be abandoned. All HCWs should be retested with IGRA if a new routine screening is scheduled. Once again, this will spare radiograph exposure for the HCW because no further medical evaluation is needed if the IGRA reverted to negative and no clinical signs of active TB are apparent. How oscillating HCWs, who change from positive to negative and back again, should be treated remains to be discussed. Assuming that recent exposure to *M. tuberculosis* is likely, a simple approach would be to rule out pulmonary TB whenever they test positive in IGRA and not to do any further medical evaluation if they test negative and no clinical symptoms are apparent. The same can be applied for HCWs falling into the borderline zone. As no further medical evaluation is needed as long as they do not show any clinical symptoms. However, as mentioned previously, data on disease progression depending on variation in IGRA are needed in the future in order to be able to derive evidence-based recommendations for the treatment of HCWs with IGRA conversion or reversion, as well as for HCWs who fall into the borderline zone with their IGRA results.

HCWs with preventive chemotherapy for LTBI in their history or HCWs with repeatedly positive IGRA in their history should not be retested in the next screening as the IGRA results would not be informative. If such a HCW belongs to a cohort with a low progression risk (*e.g.* no active TB observed in the hospital staff during the last years), a radiograph should be performed only when suspicious clinical signs are present.

Derived from the summarised evidence base above, decision trees for routine TB screening and contact tracings of HCWs using IGRA are proposed in figures 1 and 2, respectively. However, it should be borne in mind, that more research is needed in order to prove the usefulness of this approach.

Silicotuberculosis

Silicotuberculosis is present if, along with silicosis, active pulmonary TB is diagnosed at the same time. Silicosis and TB have long been known to be associated, which is why, in Germany, silicotuberculosis was included on the list of occupational diseases as far back as 1929 [33]. Dust containing crystalline SiO_2 of the kind that occurs in coal mining and other kinds of work, which is not readily soluble and is inhaled and deposited in the alveoli and airways, accumulates in the lung if the clearance rate in the respiratory epithelium is exceeded [34]. The dust particles are phagocytosed by macrophages and transported into the lung stroma. Macrophage activation and proliferation then occurs, with the creation of inflammatory and cytotoxic cytokines and cell growth factors. The phagocytosing cells are eventually destroyed by the non-degradable dust particles. A succession of new macrophages takes up the particles and continues the cycle. The result is a chronification of the inflammation process. In its further course, silicotic areas can take shape in the interstitial connective tissue, typically characterised by central hyalinisation and concentric, lamella-like strata. These changes to the pulmonary tissue and the chronic increase in macrophage activity are probably the cause of a higher susceptibility to TB infection and an increased risk of developing active TB. In recent mortality studies from Italy [35–37], Austria [38], the UK [39] and Japan [40], the standardised mortality ratio for TB in workers with silicosis was between 2.2 and 27.

In an analysis of death certificates issued between 1996 and 2006 in the USA, NASRULLAH *et al.* [41] noted a marked decline in the number of deaths due to silicotuberculosis. The first year in which silicotuberculosis was not registered as the cause of death was 2006. The authors surmise that this

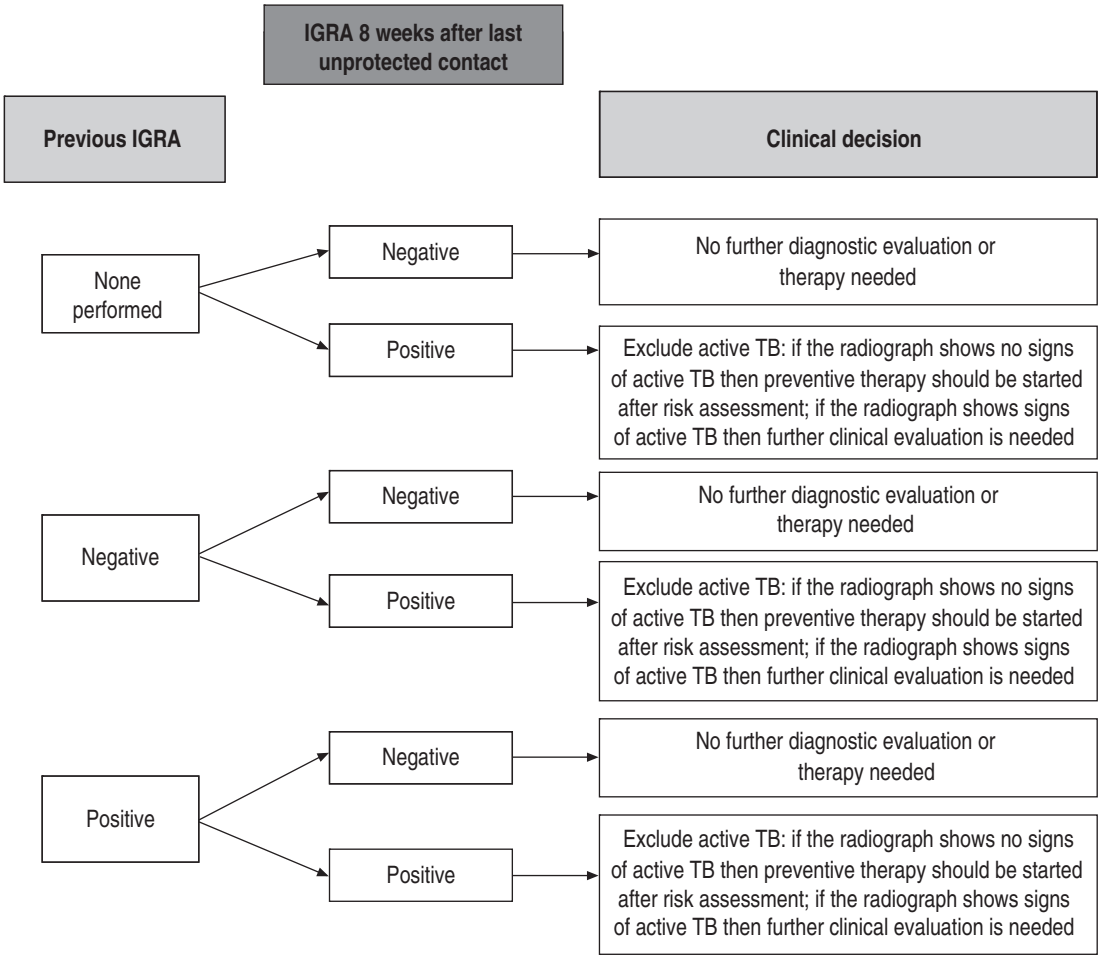
IGRA 8 weeks after last unprotected contact
Previous IGRA
Clinical decision
None performed
Negative
Positive
Negative
Positive
No further diagnostic evaluation or therapy needed
Exclude active TB: if the radiograph shows no signs of active TB then preventive therapy should be started after risk assessment; if the radiograph shows signs of active TB then further clinical evaluation is needed
Negative
Positive
No further diagnostic evaluation or therapy needed
Exclude active TB: if the radiograph shows no signs of active TB then preventive therapy should be started after risk assessment; if the radiograph shows signs of active TB then further clinical evaluation is needed
Positive
Negative
Positive
No further diagnostic evaluation or therapy needed
Exclude active TB: if the radiograph shows no signs of active TB then preventive therapy should be started after risk assessment; if the radiograph shows signs of active TB then further clinical evaluation is needed

Figure 1. Decision tree for routine screening of healthcare workers. IGRA: interferon-γ release assays; TB: tuberculosis.

was due not only to the generally low incidence of TB in the USA, but also to improved hygiene at work in connection with potential exposure to mineral dust.

In their review of TB among workers with silicosis in 2008, BARBOZA *et al.* [42] found that the risk of active TB was 2.8 to 39 times higher depending on the stage that the silicosis had reached. In 2010, VACEK *et al.* [43] reported an increased risk of TB (standardised mortality rate 21.7, 95% CI 18.37–25.56) for stone masons employed in Vermont, USA, between 1947 and 1998. The likelihood of contracting the disease was associated with the cumulative silicate dust concentration that was inhaled.

Silicosis not only increases the risk of TB, but it is also reported that TB increased the risk of silicosis in exposed workers. In 2010, ZHANG *et al.* [44] investigated the incidence of silicosis and TB in a cohort of 2,004 foundry workers in the Chinese automobile industry. The incidence of silicosis was 2.6 times higher if the workers had suffered from TB. In 2007, TSE *et al.* [45] reported swiftly progressing silicosis among 29% of 574 workers in small gold mines in China. TB in the anamnesis increased the risk of silicosis.

Silicotuberculosis in migrant workers: the African experience

In 2009, PARK *et al.* [46] reported on 513 gold miners who they followed for 1 year after they had stopped working in the gold mine. When they left the job, 27% had silicosis. The incidence of TB was 3,085 per 100,000 mine workers per year, which is a very high ratio even for Africa. These

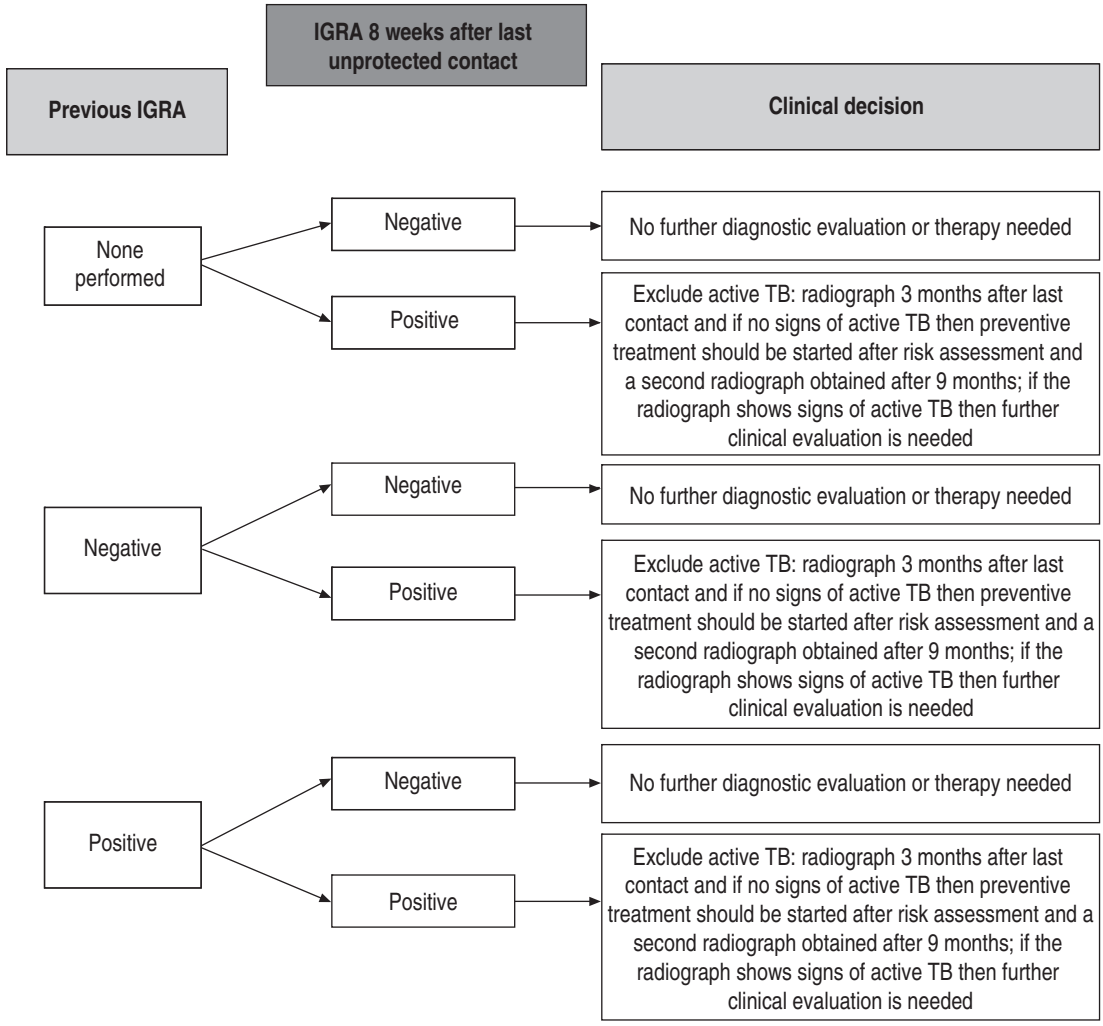
IGRA 8 weeks after last unprotected contact
Previous IGRA
Clinical decision
None performed
Negative
No further diagnostic evaluation or therapy needed
Positive
Exclude active TB: radiograph 3 months after last contact and if no signs of active TB then preventive treatment should be started after risk assessment and a second radiograph obtained after 9 months; if the radiograph shows signs of active TB then further clinical evaluation is needed
Negative
Negative
No further diagnostic evaluation or therapy needed
Positive
Exclude active TB: radiograph 3 months after last contact and if no signs of active TB then preventive treatment should be started after risk assessment and a second radiograph obtained after 9 months; if the radiograph shows signs of active TB then further clinical evaluation is needed
Positive
Negative
No further diagnostic evaluation or therapy needed
Positive
Exclude active TB: radiograph 3 months after last contact and if no signs of active TB then preventive treatment should be started after risk assessment and a second radiograph obtained after 9 months; if the radiograph shows signs of active TB then further clinical evaluation is needed

Figure 2. Decision tree for contact tracings of healthcare workers. IGRA: interferon-γ release assays; TB: tuberculosis.

figures indicate the unfortunate relationship between poor occupational health and safety, and silicosis and TB among these workers in South Africa. Therefore, improvements in health and safety and better medical care for miners in Africa are needed in order to stem the tide of a TB epidemic that is spread to the rest of the population through the migration of workers [47–49].

The reasons and the impact of TB in miners in South Africa are discussed in a review by Rees *et al.* [50]. Hundreds of thousands of males from rural South Africa and its neighbouring countries look for work in gold mines. South African law limits work contracts for migrants to 9 months, so migrants in search of employment usually return to their home countries after 9 months before getting another contract. This kind of migration is known as oscillating migration because the migrants rotate between work and their countries of origin. However, the negative influence of migration on rates of disease is not limited to miners; it has also been identified in other industries.

South African gold mines have the highest rate of TB in the world. In recent years it has increased significantly from 806 cases per 100,000 miners in 1991 to 3,821 cases per 100,000 miners in 2004. Before gold mining began, TB was infrequent in South Africa. European miners, some of whom arrived in Africa with TB, spread the disease, infecting Africans, and the migrant workers took the TB back home with them.

The incidence of HIV among South Africa gold miners rose dramatically in the 1990s. In 1987, only 0.03% of the miners had HIV. By 2000, the proportion had risen to 27%. Migrants mainly live in single-sex hostels, which is an ideal environment for the sexual transmission of diseases. The system of labour migration creates a market for needy females whose sole chance of survival is by means of prostitution. This explains why the prevalence of HIV in migrant miners is twice as high as in non-migrant miners.

HIV increases the risk of TB or of reactivating a LTBI and a swifter course of the disease. The combination of HIV and silicosis has an even greater effect on the TB due to the wide range of reciprocal effects. The partners and families of miners with both TB and HIV have an especially serious threat of infection; all the more so because miners with active TB are sent back to their home villages without it being ensured that their treatment can continue.

Migration is, for many, the sole means of survival, and industries also depend on the migrants. For logistical and political reasons, a sudden end to migration is not possible. This makes measures to reduce the negative repercussions of migration all the more important. Healthcare facilities in the returning mine workers' places of origin are overburdened and need help to diagnose and manage the occupational diseases silicosis and silicotuberculosis or migration-related HIV infection. The mining companies externalise the healthcare costs of diseases caused by the working and living conditions in their mines by sending oscillating migrants back to their countries of origin and their local medical facilities that are, for the most part, inadequately prepared for treating them. Appropriate transfer payments would be one way to not only make the mining companies pay for the costs for which they are responsible but also to create an incentive for them to improve working and living conditions in the mines. Improved accommodation, with alternatives to single-sex hostels, and a reduction in quartz dust intake are key to the solution, and the gold price on world markets must surely be sufficient to ensure the continued treatment of miners with active TB, irrespective of whether they are at the mine or staying with their families.

IGRA and silicotuberculosis

We are currently aware of three studies on the use of the IGRA in patients with silicosis. A Chinese study investigated the risk of developing active TB among silicosis patients subject to IGRA findings [51]. In this study, which used the T-SPOT®.*TB*, the IGRA predicted development of active TB better than the TST. In an earlier publication by the same working group, agreement between the TST and the IGRA was low when screening silicosis patients for TB [52]. Another study investigated the prevalence of LTBI in miners in Germany with chronic obstructive pulmonary disease (COPD) or silicosis [53]. The prevalence of positive IGRA results was high (47% or 62% depending on the IGRA used); however, no active TB was observed either at the time of the test or during the follow-up 1 year later.

Conclusion

TB in HCWs will remain an unresolved issue over the coming years even in countries with a low incidence of TB and a high average income. This will be the case all the more in low-income countries with a high incidence of TB. TB screening of HCWs using IGRA instead of the TST will be useful in countries rich in resources. However, a test that can distinguish between old and recent infection is desirable and more studies on the interpretation of IGRA results in serial testing are needed. Most valuable in this respect is data on progression to active TB depending on the variation of IGRA results. With silicotuberculosis, the social nature of TB becomes obvious. The decline in the incidence of silicotuberculosis in countries with high average income is encouraging. However, the struggle against endemic TB in African miners cannot be won without major social and political measures. Improved living conditions for migrants and the reduction of exposure to dust levels in the workplace are prerequisites for any medical success in this area.

Statement of Interest

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Chapter 17

TB in the immunocompromised host

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SUMMARY: Tuberculosis (TB) remains one of the most serious infectious complications among immunocompromised patients, such as individuals infected with HIV, solid organ or stem cell transplantation patients, patients with end-stage renal disease or individuals after tumour necrosis factor (TNF) antagonist treatment. The incidence of TB in these patient groups is in general higher compared with that of the general population and disease manifestations frequently differ from those typically found in immunocompetent patients with TB. Despite immunodeficiency as the common underlying principle, immunocompromised patient groups differ with respect to TB pathogenesis, risk of TB progression and results of immune-based testing for evidence of latent infection with *Mycobacterium tuberculosis*.

This chapter will summarise current knowledge on epidemiology, pathogenesis, diagnosis and treatment of TB in immunocompromised patients and will highlight areas in which increased knowledge is needed to improve management of TB in this vulnerable patient group.

KEYWORDS: End-stage renal disease, HIV infection, HIV/*Mycobacterium tuberculosis* co-infection, immunosuppression, tumour necrosis factor-antagonist therapy, transplantation

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The incidence of tuberculosis (TB) is higher in immunocompromised patients compared with the general population. This holds true for patients with impaired cellular immunity, such as individuals infected with HIV and recipients of solid organ transplantation (SOT) or haematopoetic stem cell transplantation (HSCT). Moreover, patients with end-stage renal failure are more prone to the development of TB due to uremia-associated immunodeficiency. Finally, the risk for progression to TB infection is increased in patients receiving tumour necrosis factor (TNF) antagonists, *e.g.* for the treatment of inflammatory bowel disease, rheumatoid arthritis and other arthritides or psoriasis. In general, this increased susceptibility emphasises the particular importance of the cellular arm of the adaptive immune response for efficient control of *Mycobacterium tuberculosis* [1, 2]. In addition, the presence of *M. tuberculosis*-specific CD4 T-cell immunity is used as a surrogate marker to assess evidence for previous contact. Consequently, a detailed knowledge of the pathomechanisms leading

to increased incidence of TB in immunocompromised patients may also contribute to a better understanding of the principles of decreased test sensitivity in this vulnerable patient group.

Epidemiology of TB in immunocompromised patients

TB results from infection with bacteria from the *M. tuberculosis* complex. Only a small proportion of those infected develop active TB but particular factors, such as the presence of immunosuppressive conditions, increase the likelihood of progression to disease. Patients with impaired immune responses are therefore more prone to develop TB than immunocompetent persons. The magnitude of the risk is likely to be dependent on the degree of immunosuppression and the endemicity of TB in the region.

HIV infection significantly increases the risk for primary and reactivation of TB. At the end of 2009, 33.3 million persons were infected with HIV, of whom 2.5 million were children (<15 years old) [3]. Approximately two-thirds of all persons infected with HIV live in sub-Saharan Africa. Between 2001 and 2009, HIV incidence had fallen by more than 25% in 33 countries, of which 22 are located in sub-Saharan Africa [3]. Several regions and countries, however, do not fit this trend. In Western, Central, and Eastern Europe, Central Asia and North America, the rates of annual new HIV infections have been stable for at least the past 5 years and there is a resurgence of HIV in several high-income countries among males who have sex with males. In Eastern Europe and Central Asia, high rates of HIV transmission continue to occur between people who inject drugs and their sexual partners [3]. If co-infected with *M. tuberculosis*, people living with HIV have a risk of TB infection 21–34 times higher than those who are HIV negative [4]. In 2010, there were an estimated 8.8 million incident cases of TB globally, of which 1.1 million (13%) were among people living with HIV [4]. An estimated 1.4 million deaths occurred among TB patients in 2010, 0.35 million being HIV positive [4, 5].

Transplantation of organs and stem cells has steadily increased during recent decades. It has been estimated that the SOT population have a 20–74 times higher risk of TB than the general population [6–9]. This risk is dependent upon the endemicity of TB in the region, the organ transplanted and the level of immunosuppression. The prevalence of TB among SOT recipients in most developed countries is 1.2–6.4%, while that in highly endemic areas has been reported to be up to 15% [6, 8]. TB incidence is particularly high among lung transplant recipients, as the receipt of a pulmonary graft instead of another organ increases the risk of developing TB up to 5.6-fold [10]. Generally, TB cases occur in the first 6–9 months after transplant, with the exception of renal transplant, where onset usually occurs later [6, 8]. Mortality is 10 times higher than in the general population (range from 19–40%) [6]. More than half (57–83%) of the mortality is attributable to TB [6–8]. This high rate of mortality can result from the delay in the diagnosis and increased incidence of disseminated disease. Developed regions report a TB frequency of 0.4 to 2.2% in HSCT recipients [6, 11]. As expected, these values are higher in high-incident areas (1–16%) [6], but even in low-incident countries such as the USA, the risk of TB among HSCT patients almost doubles that of the general population [6].

Patients with end-stage renal disease undergoing dialysis are 6–25 times more likely to develop TB than the general population [6, 12]. Age, unemployment, smoking, reduced body mass index, low serum albumin, ischaemic heart disease and anaemia were some of the identified risk factors for these patients for the development of TB. Mortality rate of TB in these patients is high (17–75%) [6, 12].

In the 1990s, the biological relevance of TNF in the pathogenesis of chronic non-infectious inflammation of joints, skin and gut, that affects 2–3% of the population, was confirmed [13]. By 2009, more than 2 million patients worldwide had received TNF antagonists for the treatment of inflammatory disorders such as inflammatory bowel disease, rheumatoid arthritis and psoriasis. Since the introduction of this therapy, increased rates of TB reactivation have been reported in those patients [13]. In fact, the relative risk of developing TB is 1.6–25.2 times higher in rheumatoid arthritis patients receiving TNF-antagonist therapy than in rheumatoid arthritis patients not undergoing such therapy, and individually depends on the clinical and/or geographical setting and

the TNF antagonist used [14–20]. Active TB in these patients usually results from reactivation of a latent infection shortly after the beginning of TNF-antagonist therapy. In addition, in countries with high incidence of TB, cases caused by new infection are particularly frequent [21–23].

Pathomechanisms of impaired TB control in immunocompromised patients

As is evident from the increased incidence of TB in immunocompromised patients, immunosuppressive conditions are known to favour progression towards TB upon primary infection with *M. tuberculosis* and reactivation from latency. In general, more severe courses of primary infections occur that are linked to an uncontrolled bacterial growth in the presence of a weakened immune system. Likewise, reactivation from a non-symptomatic well-controlled latent infection is favoured in any condition of immunodeficiency. As with immunocompetent individuals, latent infection with *M. tuberculosis* in immunocompromised patients is considered as a spectrum of clinical stages with cyclic changes between bacterial replication and immune containment [24], where immunodeficiency may shift the balance towards uncontrolled replication. Although impaired immunity is the common mechanistic basis for increased incidence of active TB in immunocompromised patients, the underlying pathomechanisms and clinical presentations differ depending on the type of immunodeficiency. As these differences also affect the magnitude and functionality of specific immunity towards *M. tuberculosis*, this will also have a variable impact on the results of immune-based testing in immunocompromised patients.

The immune response towards *M. tuberculosis* is profoundly altered in individuals co-infected with HIV, and susceptibility towards TB inevitably increases with decreasing levels of CD4 T-cells. As with other immunodeficiencies, progression from infection to primary TB is accelerated in severely immunocompromised patients and progressive immunodeficiency in HIV-infected individuals is more frequently associated with extrapulmonary or disseminated disease [5]. As CD4 T-cells play an essential role in stabilising granulomas by restricting *M. tuberculosis* to a limited number of infected macrophages, a loss of CD4 T-cells in latently infected individuals gradually disrupts this balance in favour of increased bacterial replication. Nevertheless, contrary to the generally higher bacterial burden [25], rates of sputum smear-positive diagnoses are lower, which indicates that *M. tuberculosis* occupies distinct tissue niches in HIV-infected individuals [2]. It is worth noting that the restoration of CD4 T-cells upon antiretroviral treatment (ART) in HIV/*M. tuberculosis* co-infected individuals may lead to an immune reconstitution inflammatory syndrome (IRIS) that is thought to be due to a pathological response of *M. tuberculosis*-specific T-cells [26], probably in a poorly inflamed environment with a high bacterial load [27, 28]. Interestingly, *M. tuberculosis* prevalence may critically determine maintenance of specific immunity in HIV-infected individuals with latent infection. Unlike in other immunodeficiencies, such as in transplant recipients or patients with end-stage renal disease, *M. tuberculosis*-specific immune responses in asymptomatic HIV-infected individuals in low-prevalence regions are lost, whereas specific T-cell immunity is maintained in HIV-infected individuals in high-prevalence regions and is indistinguishable in magnitude from that of healthy individuals with latent infection [29]. This indicates constant skewing of specific T-cell immunity toward environmental antigens in HIV-infected individuals.

The increased incidence of TB in recipients of solid organs or stem cell transplants is primarily due to treatment with immunosuppressive drugs that either deplete T-cells or affect their functionality, such as the ability to produce cytokines or to proliferate [6]. TB in patients after transplantation may either result from reactivation of a latent infection of the recipient and/or the donor or from *de novo* infection in the face of a weakened immune system [6]. Despite this theoretical stratification, the precise assignment to any of these scenarios is difficult in clinical practice.

The mechanistic basis for an increased incidence of TB in patients with end-stage renal disease is poorly defined. The state of uremic immunodeficiency is associated with an increased

pro-inflammatory response [30, 31] and with a decreased functional capacity of monocytes and monocyte-derived dendritic cells for stimulation of antigen-specific T-cells [32–34]. Both processes may in part be related to a poor nutritional status and decreased levels of vitamin D in patients with end-stage renal disease, which were shown to mediate immune dysfunction and misdirected inflammatory responses [35]. Together with recent findings on the pathophysiological role of vitamin D in the interferon (IFN)-γ-mediated control of *M. tuberculosis* [36], decreased levels of vitamin D may at least in part contribute towards increased susceptibility to TB in this patient population.

Patients receiving TNF-antagonist treatment have an increased susceptibility for progression towards TB and most cases occur due to reactivation from latency [14, 37]. TNF is a pleiotropic cytokine that contributes to host immunity to *M. tuberculosis* and other intracellular bacteria [38, 39], and TNF-antagonist treatment is associated with a disorganisation of granuloma integrity [37].

Diagnosis and clinical presentation of active TB in immunocompromised patients

The diagnosis of TB in immunocompromised hosts can be challenging as clinical symptoms that are related to a T-helper cell (Th) type 1 immune response (*e.g.* fever) and correlates of inflammation (*e.g.* opacities on chest radiographs) may be absent or diminished [40]. However, as a general rule, most patients with TB are symptomatic irrespective of the immune status. Cough, fever, night sweats and weight loss are the most common clinical symptoms. In immunocompromised hosts, extrapulmonary manifestations of TB are more frequent and cerebral, osteoarticular or abdominal abscesses should prompt for TB to be included among the possible causes in the differential diagnosis.

The clinical suspicion of TB is usually raised by the presence of typical signs and symptoms and by abnormal imaging studies. Suspects of pulmonary TB should be evaluated by at least two morning sputum examinations for the presence of acid-fast bacilli (AFB). Although in most suspected TB cases in immunocompromised hosts from Europe, AFB will not be detected on sputum smears, approximately 50% of adult patients with active TB will have AFB in one of two sputum smears. When available, *M. tuberculosis*-specific nucleic acid amplification techniques (NAATs) should be performed on at least one sputum sample. The latest generations of tests (*e.g.* GeneXpert®; Cepheid, Sunnyvale, CA, USA) have a diagnostic sensitivity of >95% in AFB sputum smear-positive patients and a diagnostic specificity of >98% [41]. However, in a recent study in HIV-seropositive patients with TB and negative AFB sputum smears, the diagnostic sensitivity of the GeneXpert® test was only 55% [42]. Repeating the NAATs examination on at least two additional sputum samples increased the likelihood of a positive test result in TB suspects [42]. Consequently, the diagnosis of TB can readily be established if AFB can be seen on sputum smear microscopy; however, the diagnosis is still uncertain if no AFB are seen on sputum smears, even if the NAATs shows a negative result. In this situation, bronchoscopy is indicated to obtain bronchoalveolar lavage (BAL) for microscopy, NAATs and culture, and transbronchial biopsies for a histopathological examination plus NAATs and culture [43]. Although the yield of bronchoscopy does not increase substantially for the diagnosis of TB when compared with induced sputum, bronchoscopy is essential to identify a number of important differential diagnoses, *e.g.* sarcoidosis, bronchogenic carcinoma, cryptogenic organising pneumonia (COP), or nonspecific interstitial pneumonitis (NSIP).

While immunodiagnosis using the tuberculin skin test (TST) or interferon-γ release assays (IGRAs) performed on peripheral blood cells has little role in the diagnosis of active TB, especially for immunocompromised patients, local diagnosis of specific immunity from extrasanguinous fluids by an *M. tuberculosis*-specific ELISPOT assay may be considered in TB suspects with negative AFB sputum smears and negative sputum/BAL NAATs results [44, 45]. In this situation, a relative increase in the number of *M. tuberculosis* specific lymphocytes in the BAL compared with the peripheral blood is very suggestive of active TB, whereas a negative *M. tuberculosis* specific ELISPOT

from BAL is only rarely found in active TB [45, 46]. Following a clinical algorithm in the diagnostic evaluation of TB suspects will help to identify the vast majority of patients with active TB within the first week of admission to a hospital [40].

Diagnosis of latent infection with *M. tuberculosis* in immunocompromised patients

Latent TB infection (LTBI) with *M. tuberculosis* is defined by the presence of a positive immune response in the TST and/or the absence of active TB in an *ex vivo* immunodiagnostic test performed on peripheral blood. *In vitro* tests include the ELISA-based QuantiFERON® TB Gold-In-Tube test (Cellestis, Hilden, Germany), the ELISPOT-based T-SPOT®.*TB* test (Oxford Immunotec, Abingdon, UK), or tests that rely on the detection of *M. tuberculosis*-specific immunity using flow cytometry. While ELISA and ELISPOT assays are commercially available, the use of flow cytometry is currently limited to research settings for the most part. Assay characteristics of *ex vivo* assays in relation to the TST are summarised in table 1.

Up until now, knowledge on both the negative and positive predictive values (PPVs) of IGRAs for progression towards TB has been limited. In general, the indirect immune-based definition of LTBI is only a proxy for a "true" latent infection with viable *M. tuberculosis*. Since the *M. tuberculosis*-specific memory T-cell responses identified with these methods do not discriminate between individuals with "true" LTBI, active TB or successfully treated TB, it is unclear which proportion of healthy individuals with the presence of adaptive *M. tuberculosis*-specific immune responses are indeed latently infected with viable *M. tuberculosis* [47]. Only "truly" latently infected individuals are at risk of progressing to active TB. Ideally, in the absence of more specific tests for diagnosis of individuals with the potential for progression, immunodiagnostic testing should be restricted to well-defined risk groups to identify those with the highest risk for the development of TB (fig. 1). As a rule, all candidates for immunodiagnostic testing for LTBI should accept preventive chemotherapy against TB in case of a positive test result prior to testing (intention to test is intention to treat). IGRA testing is preferable to TST because of operational advantages, including internal positive controls, and possibly superior PPV in individuals with advanced immunodeficiencies. As the decisions for preventive chemotherapy depend at large on the results of immunodiagnostic testing, the predictive values of positive and negative test results in each specific population of immunocompromised individuals and in regions of different TB prevalence need to be considered before a decision for testing is made (fig. 1).

Among the different groups of patients with immunosuppression, HIV-seropositive patients, patients receiving immunosuppressive drugs, patients with chronic renal failure and candidates for TNF-antagonist therapies are currently considered to be candidates for testing and treatment. However, the relative risk for the development of TB in immunocompromised individuals varies and has recently been described as 9.5–9.9% in patients with advanced HIV infection, 2.8% in patients taking >15 mg prednisolone equivalent per day, 2.4% in patients with chronic renal failure and 2.0% in candidates for TNF-antagonist therapies [48]. In addition, these risk estimates strongly depend on the overall prevalence of TB.

It has recently become apparent that the currently used immune-based approaches to identify individuals with LTBI are limited by the fact that the proportion of positive test results differs in the various immunocompromised patient groups. It is noteworthy that the percentage of positive test results in individual patient groups does not directly correspond to the estimated risk for progression towards TB. In a large European study, the probability of a positive result in a *M. tuberculosis*-specific immunodiagnostic test was substantially higher in patients with end-stage renal disease compared with patients with HIV infection [49], despite the substantially higher relative risk for TB in HIV-infected patients. This indicates that a positive *M. tuberculosis*-specific immunodiagnostic test in an HIV-infected individual is much more predictive for the development of TB compared with that of a patient with renal failure. As the frequency of positive test results of the TST, ELISA, ELISPOT or flow

cytometry exceeds 25% in European patients with chronic renal failure [49, 50], universal testing and consequent preventive treatment of all patients with renal failure is probably ineffective, whereas it may be highly effective to prevent TB in patients with HIV infection in Europe. Thus, although a large body of data is available on the performance of immunodiagnostic assays in risk groups of patients with immunodeficiencies, their PPV for progression towards TB in the various groups of immunocompromised patients are still poorly defined. Until they are better defined, the general acceptance and effectiveness of testing and preventive chemotherapy for LTBI remains suboptimal in Europe and elsewhere [51].

Prevention and treatment of TB in immunocompromised patients

Prevention of TB

Treatment of LTBI is the mainstay of TB prevention in immunocompromised individuals. However, avoidance of contact with infectious TB cases (*i.e.* smear-positive pulmonary TB patients) is also important in high-incidence countries, and pulmonary TB suspects and cases should be isolated from other patients in outpatient and inpatient services caring for immunocompromised individuals. The drug regimens used for treatment of latent infection with *M. tuberculosis* in immunocompromised patients are summarised in table 2.

Indications for treatment to reduce the risk of future TB in immunocompromised patients would include any of the following criteria, after exclusion of active TB disease [6, 14, 59]: 1) positive

Table 1. Immunodiagnostic tests to analyse an immune response towards *Mycobacterium tuberculosis*

	Tuberculin skin test	IGRA		
		ELISPOT	ELISA	Flow cytometry
Commercially available	RT-23, Biocine, *etc.*	T-SPOT®.*TB*	QuantiFERON® TB Gold In-Tube	In house assay
Antigens	PPD	Peptides from CFP-10 and ESAT-6	Peptides from CFP-10, ESAT-6 and TB7.7	Whole antigens or peptides from CFP-10, ESAT-6, PPD
Substrate	Skin	PBMC	Whole blood	Whole blood or PBMC
Time required for the result	48–72 hours	20 hours incubation and 3 hours for final results	20 hours incubation and 3-4 hours for final results	6 hours stimulation and 2 hours for final results
Cells involved	Neutrophils, CD4 T-cells, CD8 T-cells migrating into the skin	CD4 T-cells in the wells	CD4 T-cells in whole blood tubes	CD4 T-cells in whole blood tubes
Cytokines involved	IFN-γ, TNF-α, TNF-β	IFN-γ	IFN-γ	IFN-γ or others
Read-out units	Millimeters	IFN-γ spot forming cells	Units per mL	Percentage of CD4 T-cells
Outcome measure	Level of induration	Number of IFN-γ producing T-cells	Plasma concentration of IFN-γ produced by T-cells	Cytokine-producing cells after intracellular accumulation
Effect of immunodeficiency	Significant	Moderate/low	Moderate/low	Moderate/low
Specific for *M. tuberculosis*	No (confounded by BCG vaccination)	Yes	Yes	Yes

IGRA: interferon (IFN)-γ release assay; PPD: purified protein derivative; CFP: culture filtrate protein; ESAT: early secreted antigenic target; PBMC: peripheral blood mononuclear cells; TNF: tumour necrosis factor; BCG: bacille Calmette–Guérin. T-SPOT®.*TB* is manufactured by Oxford Immunotec, Abingdon, UK. QuantiFERON® TB Gold In-Tube is manufactured by Cellestis, Hilden, Germany. Reproduced and modified from [47] with permission from the publisher.

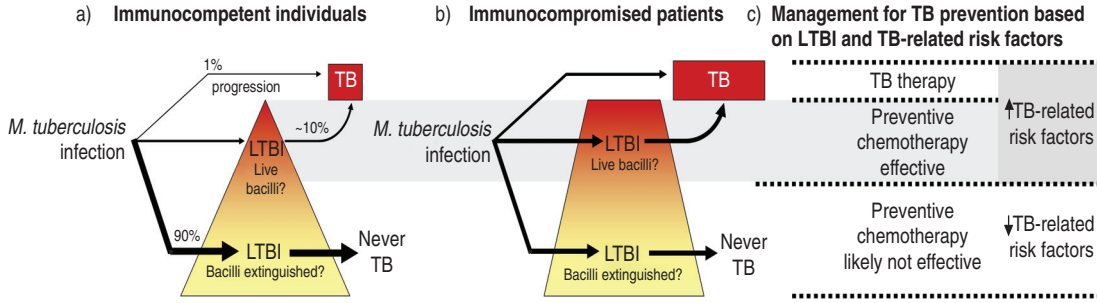

Figure 1. Implications of latent tuberculosis infection (LTBI) testing for progression towards tuberculosis (TB). a) After infection with *Mycobacterium tuberculosis*, a smaller percentage of individuals progress towards active TB, whereas the majority of individuals remain in the clinically inapparent state of latent infection with *M. tuberculosis*, which is defined as a state with detectable immunity in the absence of clinical symptoms. Of those, only approximately 10% of individuals, presumably those who harbour viable bacilli, progress towards TB within the next 1–2 years. b) In the same situation, the percentages of individuals with primary TB and of individuals with progression from LTBI towards TB is higher in immunocompromised patients. The largest fraction of immunocompetent and immunocompromised individuals with LTBI will never develop TB, presumably as bacilli are largely extinguished. c) When using a management strategy relying on universal screening, preventive chemotherapy is likely less effective unless combined with assessment of additional TB-related risk factors. The presence of these risk factors increases with rising TB prevalence, which necessitates management strategies adapted to the general TB prevalence in the respective region/country.

immunodiagnostic test (including TST $\geqslant$5 or 10 mm, depending on the risk group or IGRA); 2) chest radiograph signs of TB sequelae with no or insufficient previous TB treatment; 3) recent close contact with an infectious case (*e.g.* smear-positive pulmonary TB) in severely immunocompromised patients and children less than 5 years of age irrespective of TST or IGRA test results.

Individual national guidelines vary considerably and variations are largely based on the prevalence of TB [6, 14]. As exemplified in the transplant setting, there is evidence for universal administration of LTBI treatment without prior testing in all transplant recipients in high-incidence countries [55]. However, in low-incidence settings, expert opinion recommends LTBI treatment in transplant recipients with positive immunodiagnostic tests and at least one other risk factor (*i.e.* born in a high-incidence country, recent exposure to an infectious TB case, *etc.*) [6]. Based on the low PPVs of TST and IGRAs for progression towards TB, universal screening and treatment of all individuals with positive testing will result in a considerable extent of overtreatment, especially in low-incidence settings. Consequently, there is an urgent need for improved screening strategies with higher PPVs that are adapted to the type of immunodeficiency and to the overall prevalence of TB.

Treatment of TB

The standard treatment regimen for active TB (isoniazid, rifampin, pyrazinamide and ethambutol for 2 months (2HRZE) + isoniazid plus rifampin for 4 months (4HR)) is recommended as a first choice treatment in new TB cases including immunocompromised patients [60]. Intermittent dosing in HIV-infected individuals has been associated with increased relapse rates and increased rifampicin resistance in patients with treatment failure [61, 62], although the evidence for intermittent treatment is limited and of poor quality [63]. A trial is under way comparing intermittent *versus* daily dosing in HIV-infected individuals [64]. Consequently, until further evidence is available, daily dosing is strongly recommended in HIV-infected and other immunocompromised patients with TB, at least during the intensive phase [60, 65]. A longer duration of the continuation phase might be more efficient in HIV-infected individuals [61, 62] and other immunocompromised individuals, but available data are limited and a formal recommendation cannot be drawn. Longer duration of the continuation phase is recommended in

immunocompromised patients with pulmonary TB and cavitation on initial chest radiograph and/or positive cultures at 2 months of treatment [6, 65], and also in central nervous system and osteoarticular TB [65].

Administration of rifampicin (and to a lesser extent of rifabutin) reduces serum levels of immunosuppressive drugs used in transplant recipients (tacrolimus, cyclosporine, rapamycin, corticosteroids) [6] and antiretroviral drugs for HIV infection (mostly of nevirapine) [66]. However, administration of these drugs is usually mandatory for graft survival in transplant recipients and for increased survival and reduced viral recurrences in HIV-infected patients. The use of a rifamycin-containing regimen in transplant recipients is possible if the dose of corticosteroids is doubled and the dose of immunosuppressive drug (cyclosporine, tacrolimus or rapamycin) is increase by three-fold, and then adjusted accordingly to obtain therapeutic serum levels. Dosages should be reverted to previous regimens at the end of rifampicin treatment [6]. In HIV-infected patients, an ART regimen containing efavirenz should be given instead of nevirapine [66]. Rifabutin can be used instead of rifampicin in transplant recipients [6] and also in HIV-infected individuals. The use of a rifamycin-free regimen is possible in less severe cases; however, the duration of treatment is considerably longer (at least 12–18 months) and its efficacy is unknown.

Apart from direct treatment with anti-TB drugs, improvement of the immune status is a recommended complementary approach in immunocompromised patients. Starting ART during TB treatment has been shown to improve survival and reduce TB recurrence in HIV-infected patients with active TB and to reduce *M. tuberculosis* transmission; as opposed to starting ART after TB treatment has ended [67]. Starting

Table 2. Drug regimens for treatment of latent infection with *Mycobacterium tuberculosis* in immunocompromised individuals

Drug regimen	Evidence in HIV infected [52, 53]	Evidence in other immunocompromised states	Comments
Isoniazid 5 mg·kg^{-1} in adults and 10 mg·kg^{-1} in children [54] daily (maximum 300 mg) for 9–12 months	TB risk: RR 0.36 (95% CI 0.22–0.61) Fatality: RR 0.74 (95% CI 0.55–1.00)	Renal transplant recipients [55], liver transplant recipients [56], TNF-antagonist treatment [57]	1st choice
Rifampicin 10 mg·kg^{-1} in adults and 15 mg·kg^{-1} in children [54] daily (maximum 600 mg) for 4 months	Limited data	None	Drug interactions, concern about developing resistance
Isoniazid 5 mg·kg^{-1} daily (maximum 300 mg) + rifampicin 10 mg·kg^{-1} daily (maximum 600 mg) for 3 months	TB incidence: RR 0.41 (95% CI 0.21–0.81) Fatality: RR 0.69 (95% CI 0.50–0.95)	None	Might be preferred in HIV-infected individuals [53]
Rifampicin 10 mg·kg^{-1} daily (maximum 600 mg) + pyrazinamide 20 mg·kg^{-1} daily (maximum 2 g) for 2 months	TB risk: RR 0.54 (95% CI 0.34–0.86)	None	Largely abandoned due to hepatotoxicity
Isoniazid 900 mg + rifapentine 900 mg once weekly for 3 months	None	None	Evidence in immunocompetent individuals [58], not recommended in immunocompromised patients due to lack of data

TB: tuberculosis; RR: relative risk; TNF: tumour necrosis factor.

ART in the first month of TB treatment has a similar efficacy to starting in the third month (*i.e.* after the intensive phase), but is associated with increased incidence of IRIS and of adverse drug events, except in patients with CD4 T-cell counts of <50 μL^{-1}, where the benefit of starting ART in the first month outweighs the risks of IRIS and adverse events [28, 68, 69]. Consequently, ART should be started after 8 weeks of TB treatment (*i.e.* end of intensive phase) and as soon as possible in patients with very low CD4 T-cell counts [28, 70]. In patients with other immunodeficiencies, attempts of restoring immune status may include stopping TNF-antagonist treatment and/or reducing the level of immunosuppressive drugs in transplant recipients with severe TB and non-vital grafts.

IRIS is a paradoxical worsening of symptoms with fever, cough, lymph node enlargement or roentgenographic abnormalities within the first 3 months of TB treatment initiation in immunocompromised hosts [71, 72]. It occurs in approximately 15% of HIV-infected TB patients starting ART, and around 3% of affected patients die [73]. It can also occur in SOT recipients [74] and after TNF-antagonist treatment discontinuation [75]. Prednisone 1.5 $mg \cdot kg^{-1} \cdot day^{-1}$ for 2 weeks followed by 0.75 $mg \cdot kg^{-1} \cdot day^{-1}$ for 2 weeks is efficient in IRIS secondary to ART [76]; systemic steroids are the most commonly employed effective drugs in IRIS irrespective of its cause [77].

Conclusion

Immunocompromised hosts are at increased risk of the development of TB if they are recent contacts of patients with TB, especially those with positive AFB sputum smears. Currently, the best prognostic tests to estimate the future risk for the development of tuberculosis are *M. tuberculosis*-specific immunodiagnostic assays, the TST and IGRAs. The negative predictive value of these tests is extremely high in immunocompetent contacts, but evidence in immunocompromised patients is still lacking. As with immunocompetent individuals, the PPV of these tests for the development of TB is low and of limited use to specifically identify those who will develop TB. Thus, improved diagnostic tests and integrated strategies are needed to identify immunocompromised individuals at risk of the development of TB and to guide decisions for preventive chemotherapies.

In contrast with immunocompetent individuals, the diagnosis of TB can be more challenging in immunocompromised hosts as clinical signs and symptoms may be subtle and extrapulmonary involvement is more frequent. TB is the leading cause of mortality in HIV-infected patients worldwide and should always be considered in the differential diagnosis of respiratory diseases, meningitis or abscesses in immunocompromised patients.

Statement of Interest

M. Sester has received speaking honoraria from Roche Diagnostics and Cellestis and acts as a consultant for Genentech. She has received a patent entitled "*In vitro* process for the quick determination of a patients status relating to infection with *Mycobacterium tuberculosis*". She has performed studies in which part of the kits were provided free of charge by Cellestis and Oxford Immunotech. D. Bumbacea's travel to one Global IGRA Symposium was funded by Ewopharma. C. Lange has participated in immunodiagnostic studies where test kits have been provided free of charge by the companies Cellestis and/or Oxford Immunotec.

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Chapter 18

The WHO strategy for TB control and elimination

Marina Tadolini and Giovanni Battista Migliori

SUMMARY: Tuberculosis (TB) remains among the leading causes of death among treatable infectious diseases after HIV/AIDS, despite the existence of a cost-effective strategy for its prevention and control.

This chapter will discuss the existing strategies developed to achieve the projected goals of TB control by 2015 and elimination by the year 2050, as advocated by the Millennium Development Goals (MDGs) and the Stop TB Partnership, as well as the major threats the international community is presently facing in this respect.

After introducing the key definitions and concepts relevant to TB control and elimination, the history and content of the five core pillars of the DOTS (directly observed therapy, short course) strategy and the six elements of the Stop TB Strategy will be described, as they represent the World Health Organization (WHO)-recommended strategy endorsed by Member States. At present, as the main priorities are represented by control of multidrug-resistant (MDR)-TB and TB/HIV co-infection, the chapter will discuss the core public health interventions available on this domain, based on the available evidence. Finally, the perspective of TB elimination will be critically discussed.

KEYWORDS: Control, elimination, extensively drug-resistant tuberculosis, multidrug-resistant tuberculosis, tuberculosis

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Although more than 130 years have passed since the discovery of *Mycobacterium tuberculosis* and more than 60 years have passed since the discovery and use of the first anti-tuberculosis (TB) drugs and despite the existence of a cost-effective strategy for its prevention and control, TB remains one of the leading causes of death among treatable infectious diseases after HIV/AIDS [1–6]. In low- and middle-income countries, TB represents a major and still neglected cause of death and disability, particularly in young adults. The loss of thousands of people in their productive age has a considerable impact in countries affected by poverty, hunger and other deadly infectious diseases, as well as the current financial crisis and military conflicts [7]. Moreover, the growing epidemics represented by multidrug-resistant (MDR)- and extensively drug-resistant (XDR)-TB, HIV infection and TB in prisons, in addition to factors such as external

and internal migration, challenges in health systems and TB social determinants in health, substantially affect the global TB burden and represent the major threats to TB control [7].

Aim of the chapter

The present chapter will discuss the existing strategies developed to achieve the projected goals of TB control by 2015 and elimination by the year 2050, as advocated by the Millennium Development Goals (MDGs) and the Stop TB Partnership, as well as the major threats the international community is presently facing in this respect.

Definitions

Annual risk of TB infection: probability that an individual will be infected or re-infected by tubercle bacilli in a calendar year [8, 9].

Latent TB infection (LTBI): a latent infection with *M. tuberculosis* complex (or LTBI) is a subclinical infection with tubercle bacilli, without any clinical, bacteriological or radiological evidence of manifest disease. Typically, this is an individual who has a positive tuberculin test and normal chest radiography. LTBI usually results from contact with an infectious case of TB [8–10].

Tuberculosis (TB): TB is defined as the clinical, bacteriological and/or radiographic manifestation of disease caused by *M. tuberculosis* [8–10].

Case detection rate or ratio (CDR): for a given country and year, the CDR is defined as the number of new and relapse TB cases that were diagnosed and notified by National Tuberculosis Programmes (NTPs) divided by the estimated incident cases of TB that year. The CDR is expressed as a percentage [1, 11]. The term ''rate'' is used in the World Health Organization (WHO) Global Report, from where the definitions were derived. As this term has been used historically in TB guidelines and documents, it has been maintained in the present chapter and also in the following definitions [1, 11].

TB incidence rate: the proportion of new and recurrent cases estimated in 1 year among total mid-year population per 100,000 population [1, 11].

TB notification rate: the proportion of new and recurrent cases notified in 1 year among total mid-year population per 100,000 population [1, 11].

TB mortality rate: according to the latest revision of the international classification of diseases (ICD-10), TB mortality rate is the proportion of deaths caused by TB in HIV-negative persons in 1 year among the total mid-year population per 100,000 population. TB deaths among HIV-positive persons are classified as HIV deaths in ICD-10 [1, 12].

TB prevalence rate: the proportion of TB cases (all forms) estimated in 1 year among the total mid-year population per 100,000 population [10, 11].

Pan-susceptible TB: TB caused by *M. tuberculosis* strains susceptible to all first-line anti-TB drugs [11].

Multidrug-resistant TB (MDR-TB): TB caused by *M. tuberculosis* strains resistant to at least the two first-line anti-TB drugs isoniazid (H) and rifampicin (R) [10, 12, 13].

Extensively drug-resistant TB (XDR-TB): TB caused by *M. tuberculosis* resistant to HR plus any fluoroquinolone, and at least one of the three following injectable drugs: capreomycin, kanamycin or amikacin [13–15].

IPT: isoniazid preventive therapy. This is the treatment of LTBI to reduce the risk of progression to clinical disease [16].

3Is: the 3Is strategy includes: 1) intensified case-finding; 2) IPT; and 3) infection control [16].

TB elimination: the point at which less than one infectious (sputum-smear positive) case per 1,000,000 inhabitants emerges annually in the general population or when the prevalence of TB in general population is below 1% and continues to decrease [8, 9].

The concepts of TB control and elimination

In combating TB, the logical approach is to first control its incidence and then, when this is achieved and sustained, to decrease the prevalence of its infection until its elimination [8, 9].

TB control is the strategy aimed at reducing the incidence of TB infection and, consequently, of TB disease, being based on early diagnosis and treatment of infectious cases of TB [1]. Fewer and fewer new people in the community will be exposed to the contact with the bacilli and, therefore, less people will develop the disease [8, 9].

TB elimination is an additional strategy aimed at reducing the prevalence of TB infection, based on preventive treatment of LTBI individuals. By reducing the large pool of infected individuals, future cases of TB will be prevented.

In low-TB incidence countries, the oldest generations of the indigenous population have the highest prevalence of LTBI for two main reasons: 1) having lived through times at which the risk of becoming infected was higher than it is now; and 2) having cumulatively lived longer. On the contrary, the youngest generations have a very low prevalence and risk of TB infection. Over time, if the risk of infection in the general population continues to decline, each generation will be replaced by generations with progressively lower prevalence of infection.

History of the WHO-recommended strategies

DOTS

In 1991, the 44th World Health Assembly adopted a resolution that recognised TB as a major public health problem. Two global targets were set for the year 2000: detecting 70% of infectious cases and curing 85% of them [3, 5, 17].

The DOTS (directly observed therapy, short course) strategy, which represented the internationally recommended framework for effective TB control, was launched by WHO in 1995, following the experience accumulated by Karel Styblo in the International Union Against Tuberculosis and Lung Disease (IUATLD) supported project in Tanzania. It consisted of five components: government commitment; diagnosis through sputum smear microscopy; standardised and supervised treatment; uninterrupted drug supply; and regular programme monitoring. One of the key elements of this strategy was the supervision of drug intake. In fact, as poor adherence to treatment and premature interruption of drugs contributed greatly to prolonged infectiousness and drug resistance, the newly developed strategy was centred on direct supervision of drug intake by patients [2, 3].

In 1995, only 23% of the world's population had access to DOTS, but thanks to the progressive expansion at global level, by 2005 89% of the world's population were living in areas where DOTS services were available and since its launch, more than 22 million patients have been treated under DOTS-based services [7]. However, although the implementation of DOTS helped achieve good progress, it was insufficient to accomplish the international targets of halving TB mortality and prevalence by 2015. Furthermore, the effectiveness of DOTS was questioned, particularly in areas of high HIV prevalence and in resource-poor settings [3]. This called for a revision of the strategy that would allow wider access for all infected individuals, particularly the underprivileged in the poorest countries, and became the foundation for the development of the Stop TB Strategy in the mid-2000s.

Stop TB strategy

In 2006, following intensive exploration and discussion with TB control programme managers in high-burden countries, together with partner organisations including technical agencies and donors, WHO launched the new Stop TB Strategy (table 1). The new six-point Stop TB Strategy builds on the successes of DOTS while also explicitly addressing the key challenges faced in TB control. The aim of the Stop TB Strategy is to ensure universal access to high-quality diagnosis and patient-centred treatment for all TB patients, including those co-infected with HIV and those with drug-resistant (DR)-TB. The strategy also supports the development of new and effective tools to prevent, detect and treat TB, with the ultimate aim of meeting the 2015 MDG for TB and reducing the burden of TB worldwide [2, 3].

Adequate funding has been identified as a clear indicator of commitment towards TB control. The strategic components related to early diagnosis and effective treatment of infectious cases, the backbone of TB control, have been strengthened by including new concepts over the existing frame. In particular, emphasis was given to quality bacteriology, in view of the importance of expanding culture, drug susceptibility testing (DST) and the new molecular methods, as a response to the increasing emergence of MDR-TB, the need to control TB/HIV co-infection and to manage sputum smear-negative TB cases.

The importance of strengthening laboratory networks and improving clinical management of the cases diagnosed both in the public and the private sector has been further underlined, together with the need to ensure a managerial approach to drug procurement and quality monitoring and evaluation to allow impact measurement.

Table 1. World Health Organization (WHO)-recommended Stop TB Strategy

1. Pursue high-quality DOTS expansion and enhancement
Secure political commitment with adequate and sustained financing
Ensure early case detection and diagnosis through quality-assured bacteriology
Provide standardised treatment with supervision and patient support
Ensure effective drug supply and management
Monitor and evaluate performance and impact
2. Address TB/HIV, MDR-TB and the needs of poor and vulnerable populations
Scale up collaborative TB/HIV activities
Scale up prevention and management of MDR-TB
Address the needs of TB contacts and of poor and vulnerable populations, including females, children, prisoners, refugees, migrants and ethnic minorities
3. Contribute to health system strengthening based on primary healthcare
Help improve health policies and human resource development financing, supplies, service delivery and information
Strengthen infection control in health services, other congregate settings and households
Upgrade laboratory networks and implement the Practical Approach to Lung Health
Adapt successful approaches from other fields and sectors, and foster action on the social determinants of health
4. Engage all care providers
Involve all public, voluntary, corporate and private providers through public–private mix approaches
Promote use of the International Standards for TB Care
5. Empower people with TB and communities through partnership
Pursue advocacy, communication and social mobilisation
Foster community participation in TB care
Promote use of the Patients' Charter for TB Care
6. Enable and promote research
Conduct programme-based operational research and introduce new tools into practice
Advocate and participate in research to develop new diagnostics, drugs and vaccines

TB: tuberculosis; DOTS: directly observed therapy, short course; MDR: multidrug-resistant. Reproduced and modified from [18] with permission from the publisher.

The role of addressing risk factors [19–27] and social determinants of TB has been recently re-emphasised, to ensure that prevention and care of proximate risk factors (smoking tobacco, air pollution, overcrowding, malnutrition, *etc.*) and associated social determinants (low income, education, social protection, *etc.*) which often result from global trends such as industrialisation, urbanisation, migration and financial regression are comprehensively tackled by health systems [19, 20].

Evidence exists that TB is more common among the poor and among people in lower socioeconomic groups [1], although the nature and gradient of the association between poverty and TB changes from setting to setting.

The link between TB and crowding, malnutrition, HIV and several medical conditions impairing host defence against TB are well known [1–6]. HIV increases the risk of TB more than 20-fold. Silicosis has also gained recent attention [28].

Recent meta-analyses have established that smoking, diabetes mellitus, malnutrition and alcohol abuse increase the risk of developing active TB by two to three times [2–5]. Other possible risk factors include indoor air pollution (pollutants from burning solid fuels such as coal, charcoal, wood, dung and crop residues in open fires or inefficient traditional stoves), mental illness, illicit drug use, chronic helminth infection, and a range of other chronic diseases and treatments.

Many of these factors might be relevant for TB control. Globally, it has been estimated that about 16% of incident TB cases can be attributed to HIV, while between 10% and 27% can be attributed to the less potent but more common risk factors diabetes, alcohol abuse, smoking and malnutrition [6].

However, the importance of the different risk factors varies across settings due to different prevalence of the risk factors and different interactions among the factors themselves, and more evidence is needed, particularly from low- and middle-income countries. The interest of having better evidence on social determinants to take adequate action is obvious, as this will potentially lead to a reduction in TB incidence, prevalence and mortality.

TB prevalence surveys can be used as platforms for analytical studies on the relationship between various risk factors and TB disease. This can help improve the understanding of local TB epidemiology as well as contribute to the global evidence on TB risk factors and determinants.

WHO and partners have started discussions to define the new post-2015 strategy of TB control and elimination. Initial agreement was found to be built around three core pillars, represented by: 1) intensified and innovative TB care; 2) development and enforcement of bold health system and social development policies; and 3) promotion and intensification of research and innovation.

While the first pillar will capture the core technical principles described in the DOTS and Stop TB Strategy (rapid diagnosis, screening of populations at risk, treatment and patient support including MDR-TB and TB/HIV, and treatment of latently infected individuals), the second pillar will capture the necessary policies supporting these principles (integration of health services, universal access, improved vital statistics, infection control and rationale use of quality drugs, and fight against social determinants). Finally, the third pillar will further stimulate research and rational use of new diagnostics, drugs and vaccines [29].

Targets and goals in global TB control

Overall, since the establishment of DOTS (later enhanced to the Stop TB Strategy) in 1995, 46 million people have been cured and nearly 7 million lives were saved compared with what would have happened if pre-1995 care standards had remained unchanged [1].

The new Stop TB Strategy has greatly contributed to improving global TB control over the last 10 years. Globally, the estimated case detection rate in 2010 was 65%, and the treatment success of the 2009 cohort reached 87%, the highest ever recorded [1, 7]. Although the denominator for the case detection rate is still an estimate (*e.g.* the number of incident TB cases estimated to occur),

new instruments have been developed by an *ad hoc* Task Force to improve precision of the estimates [29]. An unprecedented plan of prevalence surveys has been launched in the priority countries with the support of the Global Fund and other donors [30].

Furthermore, the case notification rate has been added as a core indicator to overcome some of the limitations affecting the case detection rate [1]. More precise estimates of the global TB burden, resulting from this survey plan will be available in 2015.

The MDGs and the Stop TB Partnership aimed to halt and start to reverse the incidence of TB, and reduce the prevalence and death rate by 50% compared to their level in 1990 by 2015, and to eliminate TB by 2050 (table 2). With improved control efforts, most regions, except Africa, are on track to achieve the MDG target of decreasing TB incidence by 2015 [1, 7]. As far as the TB mortality is concerned, deaths from TB have fallen by 40% globally since 1990, and achievement of the 50% reduction target by 2015 is likely. Despite the TB prevalence rates falling, the target of halving the rates of 1990 by 2015 is unlikely to be achieved, except in the Americas and the Western Pacific region [1].

However, elimination of TB by 2050 is not in sight with this pace of decline, although substantial progress might be achieved in the next few decades [1, 7].

Some experts argue that a broader vision is needed, and proposed additional and ambitious targets for TB: zero children dying from TB by 2015, pursuing zero infections, zero deaths and zero stigma from TB for people of all ages.

To ensure that the post-2015 targets are epidemiologically consistent and technically sound, WHO, in collaboration with the Task Force mentioned previously, is presently discussing how mortality data from vital statistics can be used to develop new indicators to complement indicators based on TB and MDR-TB morbidity. The discussion involving national programmes, technical partners and donors is ongoing.

Control of MDR-TB

The key steps in the fight against MDR-TB and XDR-TB are documented by the Global MDR-TB and XDR-TB Response Plan developed by WHO and the outcomes of the governmental conference organised in China in April 2009 to develop the Beijing Call for Action [31]. The document committed the 27 high-MDR-TB burden countries to take action on multiple fronts towards achieving universal access to diagnosis and treatment of MDR-/XDR-TB by 2015.

The recommendations for increased investment by national TB Programmes to prevent and control MDR-TB and XDR-TB are as follows [7]. 1) Preventing XDR-TB through basic strengthening and alignment of TB and HIV programmes by increasing case detection and effective treatment of drug susceptible TB. The new Stop TB strategy and the Global Plan to Stop TB [18] are the key reference documents to guide these priority interventions until the new post-2015 WHO-recommended strategy is made available. 2) Improving the management of

Table 2. Global tuberculosis (TB) control targets

UN Millennium Development Goals
2015: Goal 6: combat HIV/AIDS, malaria and other diseases
Target 6c: Halt and begin to reverse the incidence of malaria and other major diseases
Indicator 6.9: Incidence, prevalence and death rates associated with TB
Indicator 6.10: Proportion of TB cases detected and cured under DOTS
Stop TB Partnership
2015: Reduce prevalence and death rates by 50% compared with their levels in 1990
2050: Reduce the global incidence of active TB cases to <1 case per million population per year

DOTS: directly observed therapy, short course. Reproduced and modified from [18] with permission from the publisher.

individuals suspected to be affected by MDR- and XDR-TB through accelerated access to laboratory facilities with rapid DST testing for R and H resistance [32], conventional DST for MDR-TB cases, and improved detection of cases suspected of harbouring MDR strains both in high- and low-HIV prevalence settings. 3) Strengthening MDR- and XDR-TB management and treatment design in both HIV-negative and -positive individuals, through appropriate use of second-line drugs, patient-centred approaches and adequate support and supervision. 4) Standardising the definition of MDR- and XDR-TB, including when the evidence will allow further stratification of drug resistance beyond XDR based on increased resistance and worse outcomes [33, 34]. 5) Increasing contact tracing and screening [35, 36]. 6) Promoting healthcare worker infection control and protection, mainly (but not exclusively) in high-HIV prevalence settings [37]. 7) Implementing immediate MDR- and XDR-TB surveillance activities [38]. 8) Consolidating advocacy, communication and social mobilisation activities to inform and raise awareness about TB and DR-TB.

Control of TB/HIV

The close interaction between TB and HIV requires joint effort and effective collaboration by the two health programmes, as recommended by the WHO policy on collaborative TB/HIV activities [16], which consists of a list of 12 recommended interventions, addressing areas of mutual interest, for example early case detection, management and surveillance (table 1 in chapter 2 of this issue of the *European Respiratory Monograph* presents the WHO-recommended collaborative TB/HIV activities [39]). The policy was released in 2004 and updated in 2012 with minor modifications. The goal of the policy is to decrease the burden of TB and HIV in people affected by both diseases.

Activities are divided into three sections. The first provides directives on how health services should be organised to achieve an effective response to TB/HIV control, *i.e.* by establishing a TB/HIV coordinating body at a national and sub-national level, making joint strategic planning, establishing a surveillance system for TB/HIV co-infection, and ensuring monitoring and evaluation of TB/HIV collaborative activities.

The second set of activities directs the managers of HIV services on how to minimise the burden of TB among HIV-infected individuals, through periodical clinical screening of HIV-infected subjects for TB, early identification of signs and symptoms of TB followed by diagnosis and prompt treatment of people living with HIV affected by TB, their household contacts, groups at high risk for HIV and those in congregate settings, and starting IPT for those not affected by active TB. As far as IPT is concerned, the duration of protection depends on the duration of preventive treatment. In populations with high TB prevalence, the duration of benefit following completion of a 6-month course of IPT is limited (up to 2.5 years). This is probably due to continued exposure to *M. tuberculosis* infection [40–42]. Antiretroviral therapy (ART) has a substantial protective effect against TB at both individual and population levels, and TB protection is optimum when isoniazid preventive therapy and ART are combined.

The third component shows managers of TB services how to deal with TB cases who have HIV infection (by offering the HIV test to all TB patients and TB suspects, starting routine cotrimoxazole preventive therapy (CPT) in all HIV-infected patients with active TB disease regardless of CD4 cell count and enrolling them in ART care). Evidence from randomised controlled trials has shown reduced mortality, including in areas with high levels of antibiotic resistance, morbidity and hospitalisation stay among HIV-positive smear-positive TB patients put on CPT, regardless of CD4, with no significant increase in adverse events [43–45]. All HIV-infected individuals with active TB should receive ART as soon as anti-TB treatment is tolerated (generally within 2–8 weeks) regardless of CD4 cell count. The optimum timing of initiation of ART for TB patients diagnosed with HIV is now made on the basis of the results from three randomised controlled trials [46–48], which showed the safety and superiority of early initiation of ART. As a result, AIDS and TB programmes should ensure that TB patients diagnosed with HIV infection are offered ART as early as possible, preferably within integrated services or within TB

health facilities. Effective referral to HIV services remains an alternative but relies on a solid and efficient referral system. ART delivery programmes should be as decentralised as possible.

Standardised monitoring and evaluation of collaborative TB/HIV activities is essential to determine the impact of the activities and to ensure implementation and effective programme management. This requires the establishment of harmonised indicators and nationally standardised reporting and recording templates.

Substantial progress has been made in collaborations to control TB and HIV [1]. Worldwide, the proportion of TB patients who knew their HIV status doubled between 2007 and 2010 (from 16% to 34%); in Saharan Africa, 59% of TB patients knew their HIV status in 2010. Almost 2.3 million (60%) out of 3.9 million people enrolled in HIV care during 2010 were screened for TB, a four-time increase since 2007. However, despite recommendations that ART should be started in all HIV-infected TB patients, only 46% of co-infected patients received ART [7].

Elimination: future developments

Acceleration of the present decline of TB prevalence towards TB elimination at a global level needs significant action in the following areas.

Strengthening scale-up of early diagnosis and adequate treatment based on the Stop TB Strategy

It is crucial to strengthen the quality of implementation of the Stop TB Strategy, in particular the essential elements of TB control (DOTS), such as early detection and notification of all TB cases, as well as supervised standardised treatment (avoiding the misuse of anti-TB drugs, in order to limit the circulation of the bacilli in the community, reduce morbidity and mortality due to TB and prevent the further increase of MDR-/XDR-TB rates [2, 3, 5, 7]).

National programmes should target diagnosis and successful treatment of the vast majority (ideally 100%) of existing TB cases. Some of the missing cases are already being managed, for example, in the private sector or in public health facilities not linked to the NTPs, and therefore they are not notified. In this context, the effective engagement of all care providers in DOTS service may result in a substantial increase of case notification.

Strengthening of laboratory and radiography services is the first essential step to increase the diagnostic capacity of TB and ensure timely and accurate detection of active cases.

Active screening of TB cases at health facilities will play an important role. While in the past "active" screening (when the health system takes the initiative to investigate groups of individuals harbouring a risk of TB significantly higher than that of the general population) [8] was considered opposite to "passive" screening (when the patient takes the initiative to report to health services), these definitions should be looked at with new eyes [26].

Close contacts of known cases of TB, having a substantially increased risk of contracting the disease, should undergo active screening [35]. Other high-risk groups (including individuals affected by diabetes, smoking-related diseases, alcohol misuse and malnutrition) could further increase the yield of case-finding, although further research should establish its feasibility, cost-effectiveness and sustainability. This approach will not include mass radiography screening, a past intervention at low cost-effectiveness which was popular in the former Soviet Union [49]. To be cost-effective, active screening should target risk groups that need to be identified by NTPs through operational research studies [8]. In order to improve treatment outcomes the principles of using internationally recommended treatment regimens and quality-assured drugs should be underlined. Poor treatment adherence should be addressed by building health services around the patient while addressing all the factors (including social and economic ones) potentially preventing the patient from successfully completing their treatment.

Strengthening health system policies

To continue quality case finding and treatment, NTPs should be sufficiently funded, operate within adequate infrastructure and count on sufficient workforce to implement their plans. In spite of increased international funding for TB control [50] many challenges are still slowing down the planned achievements. In Asia, for example, the private sector is the dominant health provider, which, unfortunately, often provides substandard diagnostic and treatment services [7]. Engaging the private sector to follow the international standards can remarkably improve quality of care and cure rates, while reducing the out-of-pocket expenditure for patients [26].

In addition, as already mentioned, TB is associated with many comorbidities and social determinants [19]. Services for such TB patients are often underdeveloped in low- and middle-income countries and, as a result, TB often remains undiagnosed. Therefore, TB patients require either access to basic primary healthcare services or appropriate TB care. Within national health plans, NTPs should strengthen the collaboration with other public health programmes, to contribute to the prevention, treatment and management of these conditions.

Linking with the broader development agenda

Socioeconomic factors can affect the TB epidemic, as demonstrated by the morbidity and mortality curves in Europe, the USA and other industrialised countries [26]. Most of the risk factors for TB are associated with social conditions [20, 51]. People from the so-called low socioeconomic status are more likely to have frequent contact with infectious TB cases, live and work in crowded conditions, have lower levels of health awareness and have less access to quality healthcare, compared to those living in high-income countries. As discussed previously, HIV, smoking, alcohol abuse, malnutrition and diabetes are also more prevalent in low socioeconomic groups [52]. However, some aspects of economic development might also have a negative effect on the TB epidemic, as rapid industrialisation, urbanisation and migration can create ideal conditions for TB spreading, particularly in developing countries. It is known that TB incidence in urban areas is generally higher than in rural areas, probably due to the combination of high population density and lifestyle changes associated with urban living. Public health interventions to tackle the specific TB risk factors and high-level political decisions to reduce poverty and promote social protection, education and empowerment are mostly needed, and will be further tackled in the new post-2015 strategy.

Promotion and intensification of accelerated research action is needed to develop new technologies for prevention (new vaccines), diagnosis (rapid tests) and treatment (new drugs). Further research is crucial to further investigate the role of risk factors for TB and social determinants. Furthermore, effectiveness and cost-effectiveness of new strategies aimed at improving prevention, early case detection and treatment should guide future policies. Finally, TB prevalence surveys, TB mortality surveys and drug resistance surveys need to be carried out by further countries, particularly in Africa, to assess, in an accurate and precise way, the current TB burden and its trends. Although TB research investments have increased in recent years, from very low amounts [53], the present investments are insufficient to accelerate research for TB elimination.

Conclusions

The International Standards of Tuberculosis Care [54] document summarises the standards of care that public and private healthcare providers should follow when managing suspected or confirmed TB patients, in order to ensure an optimal prevention, diagnosis and treatment of TB. In particular, it is focused on early bacteriological diagnosis, accurate prescription of anti-TB drugs, management of vulnerable groups (*e.g.* HIV-positive individuals), and the recording and reporting of treatment outcomes.

Recently, the European Respiratory Society and the European Centre for Disease Prevention and Control jointly developed the European Union Standards for Tuberculosis Care (ESTC), which

represents an adaptation of the International Standards of Tuberculosis Care to the epidemiological scenario of the European Union/European Economic Areas [32, 55].

The new guidelines represent an important recognition that, although widely believed to be a disease of the past, TB continues to pose a significant public health threat to Europe and worldwide, underlining the need for urgent action.

A lot of effort and up-to-date actions are needed to control and ultimately eliminate TB. Every country should first of all enhance and maximise the implementation of the Stop TB Strategy, ensuring early diagnosis and proper treatment, strengthening of health system policies, promoting and intensifying research efforts, and supporting the development of new diagnostics, anti-TB drugs and vaccines. In addition, sustained political engagement and participatory involvement of all stakeholders, including health, social and economic sectors, are highly needed to alleviate poverty and other social determinants of TB to ensure equity and human right respect towards TB elimination.

Statement of Interest

None declared.

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Educational questions

1. Which one of the following statements is true? The current vaccine against TB, *M. bovis* BCG:

❑ Works well against paediatric TB. ❑ Does not work well against paediatric TB. ❑ Works well against pulmonary TB in adults. ❑ Works well against disseminating TB in adults.

2. Which one of the following statements is true? New vaccines against TB are being developed that:

❑ Primarily replace BCG. ❑ Primarily boost BCG. ❑ Have protective activity in already-infected hosts. ❑ All of these.

3. Which one of the following statements is true? Effective host defence against TB is least dependent on:

❑ CD4 and CD8 T-cells. ❑ Innate immunity. ❑ Humoral immunity. ❑ Macrophages.

4. Which one of the following statements is true? Live vaccines against TB may give rise to complicating dissemination of the injected vaccine strain in:

❑ The genetically immunocompromised. ❑ HIV-infected adults. ❑ HIV-infected infants. ❑ All of these.

Continued on next page

5. Which one of the following statements is true? TB vaccines should educate the immune system to recognise *M. tuberculosis* bacteria primarily:
❑ Inside infected B-cells. ❑ Inside infected macrophages. ❑ Inside infected lung epithelial cells. ❑ Outside infected host cells.

6. Which one of the following statements is true? The current generally accepted definition of low incidence for TB is:
❑ TB incidence <10 per 100,000 of population. ❑ TB notification rate <10 per 100,000 of population. ❑ TB incidence <20 per 100,000 of population. ❑ TB notification rate <20 per 100,000 of population. ❑ There is no current consensus.

7. Which one of the following statements is true? XDR-TB is defined as:
❑ TB caused by *M. tuberculosis* resistant to isoniazid and rifampicin, and to the anti-TB fluoroquinolones. ❑ TB caused by *M. tuberculosis* resistant to isoniazid and rifampicin, and to at least one of the second-line injectable anti-TB drugs. ❑ TB caused by *M. tuberculosis* resistant to isoniazid or rifampicin, and to any of the anti-TB fluoroquinolones, as well as to at least one of the second-line injectable anti-TB drugs. ❑ TB caused by *M. tuberculosis* resistant to isoniazid and rifampicin, and to any of the anti-TB fluoroquinolones, as well as to at least one of the second-line injectable anti-TB drugs. ❑ TB caused by *M. tuberculosis* resistant to all tested anti-TB drugs.

8. Which one of the following is not a risk factor for DR-TB in low-incidence countries?
❑ Pharmokinetic variability of anti-TB drugs. ❑ Diabetes mellitus. ❑ HIV infection. ❑ Immigration from a high MDR-TB-prevalence county to a low-incidence country. ❑ Previous TB treatment.

9. In which one of the following situations would you forgo a molecular drug-resistance test in a smear-positive patient in a low-incidence country?
❑ In an HIV patient. ❑ In an immigrant, who has already lived for 5 years in the low-incidence country, being a migrant from a high-incidence country. ❑ In a patient who will receive a rapid DST with MGIT 960. ❑ In a patient with previous TB history. ❑ None of these.

10. Which one of the following statements is true? In comparison with first-line anti-TB drugs-based treatment regimens, treatment with second-line anti-TB drugs is:
❑ More expensive. ❑ Less effective. ❑ More toxic. ❑ More expensive and less effective. ❑ More expensive, less effective and more toxic.

11. The WHO provides policy recommendations to assist national TB programmes to implement evidence-based TB diagnostic tools. Important recent WHO policy statements for TB diagnostics include which one of the following:
❑ Endorsement of the Xpert® MTB/RIF assay for frontline TB diagnosis in HIV-infected patients and patients suspected of DR-TB. ❑ A negative policy document advising against the use of current serological (antibody-detection) assays for TB diagnosis. ❑ A guideline document advising against the use of IGRAs for active TB diagnosis in low- and middle-income countries. ❑ All of these. ❑ None of these.

12. Which one of the following statements is not true? The Xpert® MTB/RIF (GeneXpert®) assay:
❑ Is a fully integrated, automated NAAT. ❑ Is able to successfully diagnose approximately 70% of smear-negative, culture-positive TB. ❑ Is able to provide DST for rifampicin and isoniazid. ❑ Can be implemented outside reference laboratories. ❑ Is able to provide a result within 2 hours.

13. Which one of the following tests would offer the fastest DST for both rifampicin and isoniazid in a smear-positive patient with suspected MDR-TB?
❑ Mycobacterial growth in tube liquid culture. ❑ GenoType MTBDR*plus*® assay. ❑ MODS. ❑ GeneXpert® MTB/RIF assay. ❑ Thin layer agar.

14. Which one of the following statements about IGRAs is not true:
❑ They are meant for the detection of latent TB infection. ❑ They are generally more specific than TST. ❑ They can be used to diagnose active TB disease in countries with a high TB burden. ❑ They are *in vitro* assays. ❑ They are generally more expensive than TST.

15. Which one of these commercial technologies is not WHO-endorsed?
❑ Xpert® MTB/RIF. ❑ Line probe assay (*e.g*. GenoType MTBDRplus® assay). ❑ Liquid culture (*e.g*. mycobacteria growth indicator tube). ❑ Urine LAM POC test. ❑ LED microscopy.

16. Which one of the following statements is true? The standard TB treatment regimen for new cases is:
❑ 4HRZE/2HE. ❑ 2HRZE/4HR. ❑ 4HR/2HRZE. ❑ 2HR/4HRZE. ❑ 6HRZE.

17. Which one of the following statements is true? Treatment of active TB with a single drug:
❑ Cannot lead to a substantial rate of sputum smear conversion. ❑ Cannot lead to a substantial rate of sputum culture conversion. ❑ Will cause a substantial rate of relapse with drug-resistant *M. tuberculosis*. ❑ Should be tried in the continuation phase of treatment when resources are limited. ❑ Is cost-effective in the long run.

18. Which one of the following statements is true?
❑ Rifampicin is currently the best available drug in treatment for TB. ❑ Rifampicin is the first choice for the treatment of latent infection with *M. tuberculosis*. ❑ Rifampicin has excellent central nervous system penetration. ❑ Rifampicin changes urine colour to blue. ❑ Rifampicin cannot be protected from acquisition of drug resistance by any other anti-TB drug.

19. Which one of the following statements is true? Treatment of EPTB:
❑ Should always be longer than for pulmonary TB. ❑ Is usually combined with surgery. ❑ Should include corticosteroids in TB pleurisy. ❑ Can be shortened to 4 months in TB osteoarthritis. ❑ Is prolonged in severe TB meningitis.

20. Which one of the following statements is true? The treatment of MDR-TB:
❑ Should include an earlier generation fluoroquinolone, if possible according to DST. ❑ Should not include amikacin, capreomycin or kanamycin because of the toxicity profile of these injectable drugs. ❑ Should not include PZA because of the likelihood of PZA drug resistance. ❑ Should include a later-generation fluoroquinolone, if possible according to DST. ❑ Should always include high-dose isoniazid to overcome the drug resistance.

21. Which one of the following statements about TB treatment is true?
❑ If rifampicin is not included in a regimen, the total treatment duration should be at least 18 months. ❑ Cycloserin and meropenem are WHO Group 4 drugs. ❑ Up to 90–95% of isoniazid-resistant *M. tuberculosis* are MDR-TB. ❑ Treatment of TB in HIV-positive patients should be at least twice as long as in HIV patients. ❑ Surgery should be avoided in the treatment of MDR-TB.

22. Which one of the following are risk factors for adverse effects during treatment of TB?
❑ Advanced age. ❑ HIV infection. ❑ Malnutrition. ❑ Advanced age and malnutrition. ❑ Advanced age, HIV infection and malnutrition.

Continued on next page

23. In the treatment of DR-TB among HIV-infected patients, additive toxicity may occur between antiretroviral therapy and anti-TB drugs with respect to which one of the following:
❑ Depression. ❑ Lipodystrophy. ❑ Dysglycaemia. ❑ Depression and dysglycaemia. ❑ Lipodystrophy and dysglycaemia.

24. Which of the following statements is not true in relation to the management of cutaneous hypersensitivity reactions?
❑ For mild skin reactions, it may be possible to continue anti-TB drugs with symptomatic treatment and close clinical monitoring. ❑ After resolution of skin rashes, serial reintroduction of drugs one by one aims to identify the causative drug. ❑ During re-challenge, each drug should be reintroduced at gradually increasing doses over 1 week. ❑ Re-challenge of a strongly suspected drug should be avoided for very severe reactions. ❑ If a reaction occurs during drug re-challenge, drug desensitisation may be considered if the causative drug cannot be readily replaced.

25. Adjustment of dose and/or dosing frequency is required for which one of the following drug(s) in patients with a creatinine clearance <30 mL·min^{-1} or receiving haemodialysis:
❑ Levofloxacin. ❑ Rifampicin. ❑ Cycloserine. ❑ Levofloxacin and cycloserine. ❑ Levofloxacin, rifampicin and cycloserine.

26. Which one of the following statements regarding the use of injectables (aminoglycosides and capreomycin) is false?
❑ Nephrotoxicity of the injectables usually involves renal tubules and may present with non-oliguric acute renal failure. ❑ The serum potassium level should be monitored with prolonged use of capreomycin. ❑ Risk factors include old age, renal impairment, dehydration, high trough concentration, prolonged use, use of loop diuretics and liver disease. ❑ Injectables are absolutely contraindicated in patients on haemodialysis. ❑ None of these.

27. Which one of the following statements is true? Neuropsychiatric reactions may occur with:
❑ Isoniazid. ❑ Cycloserine. ❑ Fluoroquionolone. ❑ Isoniazid and cycloserine. ❑ Isoniazid, cycloserine and fuoroquionolone.

28. Which one of the following statements concerning the use of fluoroquionolones is false?
❑ Of the commonly used fluoroquinolones, the risk of prolonged QT interval is highest with moxifloxacin. ❑ The prolonged QT interval occurs through blockade of the voltage-gated calcium channels. ❑ Moxifloxacin should be used with caution in patients at risk of Torsades de pointes. ❑ Caution is warranted with the concomitant use of methadone, levomethadyl or other opiods. ❑ None of these.

29. Which one of the following statements regarding hepatotoxicity related to anti-TB drugs is false?
❑ Pyrazinamide appears to be the most hepatotoxic agent among the first-line drugs. ❑ Risk factors include old age, malnutrition, alcoholism, hepatitis B and C infections, and HIV infection. ❑ Aspartate transaminase elevation may indicate abnormalities in the liver, muscle, heart or kidney. ❑ There is considerable mortality risk amongst patients with concurrent elevations of alanine transaminase that are more than three times the upper limit of normal and of bilirubin more than two times the upper limit of normal. ❑ None of these.

30. Which one of the following statements regarding the epidemiology of childhood TB is true?
❑ All children diagnosed with TB should be tested for HIV infection. ❑ As children rarely transmit disease, household contact tracing is not necessary. ❑ School-age children are at the highest risk of progression from infection with *M. tuberculosis* to clinical disease. ❑ BCG vaccination has had little impact on the TB epidemic in the majority of the world; therefore, it no longer has a role to play in the prevention of TB. ❑ The majority of cases of childhood TB are microbiologyically confirmed.

31. Which of the following statements about the diagnosis of pulmonary TB in children is true?
❑ Negative cultures exclude the diagnosis of TB. ❑ Induced sputum specimens can only be used in children >7 yrs of age. ❑ A negative TST rules out disease in children. ❑ Fine-needle aspiration of cervical lymph nodes may be diagnostic in suspected TB. ❑ Chest CT is never indicated for the diagnosis of TB in children.

32. Which one of the following statements is true? In the treatment of childhood TB:
❑ Children should receive the same duration of treatment as adults. ❑ The doses of anti-TB treatment (per kg) are the same for children and adults. ❑ Children are more likely to get hepatic toxicity. ❑ In children, drug-resistant TB usually develops due to poor adherence to therapy. ❑ Children with HIV-TB co-infection and low CD4 counts should start ATT and ART concurrently.

33. The most severe form of childhood TB is TB meningitis. Which one of the following statements is true?
❑ The risk of developing TB meningitis can be up to 10% in infants compared with 1% in adults. ❑ Steroids are contraindicated for the treatment of TB meningitis. ❑ Blood brain penetration of ethambutol is better than that of isoniazid. ❑ An LP typically shows low protein and high glucose in TB meningitis. ❑ Magnetic resonance imaging of the brain is rarely helpful in diagnosis.

34. Contact tracing is very important in childhood TB. Which of the following statements are true? (Select all that apply.)
❑ The TST is a nonspecific test and the results may be influenced by BCG vaccination, non-tuberculous mycobacteria, immunosuppression and HIV. ❑ Children under 2 yrs of age should be screened clinically and radiologically for TB regardless of TST or IGRA result. ❑ A negative IGRA result rules out TB infection. ❑ BCG vaccination can cause a false-positive IGRA result. ❑ An indeterminate IGRA result in a child should be treated as a negative result.

35. Childhood TB should be suspected in which of the following clinical scenarios? (Select all that apply.)
❑ A 12 year old with a cough for >2 weeks, fever and night sweats. ❑ A 2-yr-old Somali infant admitted with fever and respiratory illness of 3 days' duration; chest radiograph shows hilar adenopathy. ❑ A 4 year old who presents with focal seizure and low-grade fever; LP is unremarkable; magnetic resonance imaging of the brain shows basal enhancement. ❑ A 6 year old with a 3-month history of weight loss and abdominal discomfort, and a 2-week history of low-grade fever. Ultrasound of the abdomen shows abdominal lymph nodes. The child's grandmother (who is from India) came to live with the family 9 months ago. ❑ A 4 year old referred from the GP with cervical lymphadenopathy of 3 weeks' duration and with no response to a 10-day course of augmentin.

36. How do IGRA differentiate between BCG vaccination, *M. tuberculosis* infection and *M. tuberculosis* disease in children? (Select all that apply.)
❑ They are the same as the TST, but are based on a blood sample. ❑ They are a test for active TB. ❑ They can distinguish between *M. tuberculosis* infection and disease. ❑ They have antigens that will elicit a response only if the person is sensitised to *M. tuberculosis* but not to BCG. ❑ They cannot differentiate between active and latent TB.

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